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(57) Abstract

A carbohydrate-based library is described which comprises a plurality of distinct sugar-containing ligands each bound to a resolvable portion of a solid support. The library is constructed by a method that includes a glycosyl bond-forming step. Libraries of differing sizes can be prepared by the method of the present invention in which large numbers of distinct species are made substantially concurrently by the formation of glycosyl bonds among many types of participants. Moreover, an assay, which allows the substantial simultaneous screening of essentially all the members of the library, is described. The isolation of novel ligands of low-affinity is thus facilitated.

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CARBOHYDRATE-BASED LIGAND LIBRARY, ASSAY AND METHOD

1. Field of the Invention

The invention relates to a library of carbohydrate-based ligands, which are bound to and presented on a solid support to permit multivalent interactions with a variety of probes having a plurality of carbohydrate binding sites. Methods of the preparation of the library, the library's characteristics and an assay for selecting particular ligand-probe interactions are also described.

2. Background Of The Invention

Carbohydrates play central roles in a wide variety of normal and abnormal biological recognition processes. Among their less benign roles, carbohydrates on cell surfaces have been implicated in chronic inflammation, in viral and bacterial infection, and in tumorigenesis and metastasis. Strategies to block the interactions between cell surface carbohydrates and their protein targets could provide an effective means of preventing, treating, or alleviating the effects of various diseases. Therefore, the identification of ligands, which bind to the protein targets better (i.e., with greater affinity) than the natural cell surface carbohydrates, would be of great interest as potential candidates for mediating biologic or physiologic processes.

2.1. Combinatorial T chniques

Although the power of combinatorial synthesis for identifying drug leads and elucidating

structure-activity relationships has been appreciated for some time, a combinatorial approach has not been successfully applied to the production and screening of carbohydrate-based ligands.

It is generally agreed that the preferred approach to the construction of compound libraries involves the synthesis of the molecules of interest on some sort of solid support. Methods to make peptides and nucleic acids on solid supports have been available for many years, and so it is not surprising that the first combinatorial libraries involved peptides and nucleic acids. In contrast, the synthesis of carbohydrates has always proved to be a more difficult task in solution, let alone on the solid phase. Consequently, the preparation of solid phase carbohydrate-based libraries has been an elusive objective. At best, prior efforts have concentrated on the preparation of carbohydrate-based libraries in solution.

Certain libraries of sugar-modified, solid support-bound peptides have been described. See, International Publication No. WO 95/18971 (13.07.95). However, only N-linked glycoside species are described. Moreover, covalent attachment of the sugar to the peptide is accomplished through an amide bond formed by the condensation of the amino group of an aminosugar with the carboxylic acid group of a resin-bound peptide. In particular, the preparation of a multitude of sugar-containing compounds, including O-linked glycoside species, bound to a solid support, in which new and distinct glycosyl bonds are formed substantially concurrently, has not been described.

2.2. Affinity-Based Assays

Typically, combinatorial strategies for identifying ligands against particular receptors are evaluated by determining if it is possible with the

particular strategy to "pull out" (i.e., to selectively locate and identify) the binding ligand from a library that contains the binding ligand along with a great many other components. In conventional assays, the binding ligand, which may be a natural or unnatural ligand of the receptor under study, must bind significantly more tightly to the receptor than most of the other compounds in the library; otherwise, the binding ligand cannot be identified easily.

Carbohydrates are unusual ligands for proteins because they are relatively hydrophilic and bind with relatively low affinity to their receptors. Accordingly, many naturally occurring carbohydrate ligands bind only weakly to their receptors. Standard *in vitro* assays show only a small difference in affinity between the natural ligand and other ligands. Hence, it is usually impossible to select the natural carbohydrate ligand for a carbohydrate-binding protein from a mixture of carbohydrates in solution.

Nature solves the problem of the intrinsically low affinity interactions of carbohydrate-based ligands and their protein receptors through polyvalency, a strategy in which a plurality of ligands are displayed on a surface (e.g., the surface of a cell). In this way, carbohydrate-binding proteins, the majority of which possess multiple carbohydrate binding sites, are able to amplify the affinity and specificity of individual carbohydrate or carbohydrate-based ligands. Several studies have provided strong evidence supporting the importance of polyvalency or multivalency in carbohydrate ligand-protein receptor interactions. Nevertheless, carbohydrate-protein interactions are typically evaluated using assays that focus on the behavior of monovalent carbohydrate ligands in solution. Evidently, it has often been assumed that the

relative affinities of monovalent carbohydrate ligands in solution correlate with their polyvalent avidities.

The unique difficulties associated with carbohydrate ligands are not limited to the problem of library screening. Despite intense interest, it has been difficult to study the relationship between the structure and function of individual cell-surface carbohydrates because conventional binding assay methods are geared toward evaluating the strength and specificity of individual binding interactions. Making individual carbohydrates and assessing their binding properties in a standard solution assay does not usually work, because, as described above, the individual binding affinities are too weak.

Moreover, the low affinity of individual carbohydrates for their receptors gives rise to low specificity, i.e., only small differences in dissociation constants exist among natural carbohydrate ligands and other potential carbohydrate ligands. This difference is usually so small that it is statistically insignificant in the absence of a very large number of experiments designed to measure binding affinities. It is possible that polyvalent binding would amplify specificity as well as affinity. Hence, it is necessary to evaluate individual carbohydrate-protein interactions in a context that allows for polyvalent binding.

The existing strategies for making polyvalent carbohydrate ligands are difficult to implement for individual carbohydrates and quite useless for the screening of compounds, because these existing strategies are synthetically cumbersome. They involve synthesizing individual carbohydrates and then coupling them to each other or to a protein or to a surface. Because the amount of time required to make a single carbohydrate ligand in solution is considerable, and even more time is required to

prepare a polyvalent presentation of the ligand, it would literally take years of effort to prepare and screen even a few dozen carbohydrates using presently available methods.

Ideally, thus, to permit screening of carbohydrate libraries containing hundreds or thousands of compounds against many biologically interesting protein targets, it is necessary to have an assay that can mimic and take advantage of the multivalent binding interactions found in natural systems. Given a polyvalent carbohydrate binding protein, the ideal assay must be capable of presenting multiple carbohydrate ligands in the appropriate spatial arrangement to permit multivalent binding. In general, one will not know the precise spatial arrangement of carbohydrate ligands required for effective polyvalent binding, and the ideal assay must, therefore, be able to present the multiple ligands with some degree of flexibility, particularly conformational flexibility. Such flexibility permits the individual ligands to orient themselves independently into the optimum position and conformation for binding, yielding ultimately the lowest-energy (and thermodynamically most favored) ligand-receptor geometry. At the same time, the carbohydrate ligands will be anchored in proximity to one another, greatly reducing the entropy loss that would otherwise weigh against the desired multivalent binding.

2.3. Lectins

Lectins are a group of naturally occurring proteins having the ability to agglutinate erythrocytes and many other types of cells. The term "lectin" may be used to designate any sugar-binding protein or glycoprotein of non-immune origin, which agglutinates cells or precipitates glycoconjugates. They are known to exhibit a variety of unusual

biological properties, including specific interactions with human blood groups, induction of lymphocyte proliferation, preferential agglutination of mouse tumor cells over cells from normal tissue, agglutination of virally or chemically transformed cell lines, mediation of nuclear envelope phosphorylation, or facilitation of bone marrow transplants in patients having severe combined immunodeficiency.

Lectins are widely distributed in nature and can be found primarily in seeds of plants, although they can also be found in roots, leaves and bark. A lectin of particular interest is that isolated from the seeds of the camel's foot tree, *Bauhinia purpurea*. This lectin, first reported in 1958, is a well-known agglutinin of human red blood cells. The specificity of this lectin for various carbohydrates has been studied extensively, and it is generally accepted that it agglutinates red cells by binding to the *O*-β-D-galactopyranosyl-(1→3)-N-acetyl-D-galactosamine group present in the mucin component of the red cell membrane. Osawa, T. et al., in *Meth. Enzym.* (1978) 50:367-372. The lectin consists of four identical subunits, each of which binds to a carbohydrate ligand, and thus this lectin is an example of a multivalent carbohydrate-binding protein (i.e., a potential probe "having a plurality of carbohydrate binding sites").

In an extensive study of the specificity of *Bauhinia purpurea* lectin for 45 different carbohydrate ligands, Wu and co-workers concluded that a β-linked, N-acetyl-D-galactosamine glycosylated at the 3-position was the primary determinant of binding. These researchers had examined the relative binding affinities of ligands having an N-acetyl glucosamine group versus an N-acetyl galactosamine and had found that the former group exhibited, at most, one-third of the activity

of the latter group of ligands. Wu, A.M. et al., in *Arch. Biochem. Biophysics* (1980) 204:622-639. Furthermore, Osawa and co-workers reported that although agglutination of red cells by this lectin could be inhibited by a glycopeptide containing Gal→GalNAc residues, agglutination was "not significantly inhibited by a glycopeptide ... which contains Gal→GlcNAc sugar sequence." Osawa, T. et al., in *Meth. Enzym.* (1978) 50:367-372.

There is a need, therefore, for a method of generating a carbohydrate library that possesses a high degree of molecular diversity and which can take advantage of the polyvalent nature of carbohydrate-binding receptors. There is also a need for a method of assaying substantially simultaneously essentially all members of such a library. Such an assay should preferably take advantage of polyvalent binding to allow for the selection and identification of low-affinity binding ligands, which would normally be undifferentiated (and remain undetected) in a solution binding affinity-based assay. The preparation of such a library and the demonstration of such an assay to locate and detect a particular member or members of the library, which bind to a multivalent probe or receptor of interest, would be a great advance in the art.

According to the present invention, a method has been developed for synthesizing and screening combinatorial libraries of polyvalent carbohydrate ligands that can be used to investigate key issues involving carbohydrate recognition. In contrast to the expectations of the state of the art, the present finding shows that there is not a good correlation between the monovalent affinities and polyvalent avidities of carbohydrate ligands. The presentation of carbohydrates on the polymer surface has a profound influence on the interaction of the ligand with the protein receptor. Furthermore, the

present invention exhibits an unanticipated degree of specificity in carbohydrate recognition and suggest that carbohydrates may play a greater function as recognition signals in Nature than has been recognized previously.

3. Summary Of The Invention

The present invention is directed to a library of carbohydrate ligands from which specific compounds can be isolated, which compounds exhibit affinity binding characteristics selective for a given receptor. In particular, a library of carbohydrate-based ligands bound to a solid support is described in which multiple copies of specific ligands are generated and presented on the surface of the solid support in a way that allows a polyvalent receptor molecule to undergo a multivalent binding interaction with a plurality of carbohydrate-based ligands. Such an approach uniquely exploits the additional binding interactions possible with multivalent receptors and enhances the probability that equally unique binding substances can be isolated from a collection of inherently low affinity binding carbohydrate-based ligands.

The preparation of carbohydrate-based ligands bound to a multivalent support is described. In particular, the present method gives rise to the preparation of a collection of distinct carbohydrate-based ligands bound to a solid support, which method includes a glycosyl bond-forming step. Remarkably, a plurality of glycosyl acceptors and glycosyl donors can be used, substantially concurrently, each combination giving rise to a newly formed glycosyl bond and, hence, a distinct carbohydrate-based ligand. The resulting library of carbohydrate substances can have a prodigious number of molecularly diverse members. What is more, the library of the invention mimics the polydentate

binding properties of naturally occurring cell-surface carbohydrates. This mimicry is surprising in that the individual carbohydrate moieties are not in a fixed geometric relationship to one another. Evidently, the preferred solid support presents a multiplicity of each distinct carbohydrate ligand in spatial proximity, but with relative geometric flexibility, so that the relative orientation of potential binding ligands of the receptor which leads to effective, selective binding is permissible.

Accordingly, what is shown herein for the first time is the preparation of a library of polydentate carbohydrate-based ligands bound to a solid support, which on screening with a given polyvalent receptor, provides a unique substance that is detected as a result of a selective recognition process operating between the chosen receptor and the uniquely suited ligand.

It is thus an object of the present invention to present an effective combinatorial chemical synthesis method for the preparation of such a library of polyvalently presented carbohydrate-based ligands.

It is also another aspect of the invention to provide an assay method for the detection, isolation and identification of carbohydrate ligands exhibiting a wide range of useful properties. Such ligands may, for example, display potential agonist or antagonist activity with respect to a given receptor, or the ligand of interest may inhibit the activity of a particular enzyme. What is more, such ligands may also form the basis of effective vaccines against disease brought on by infectious agents (e.g., viral or bacterial pathogens) or hyperproliferative conditions (e.g., malignant or non-malignant tumor growth).

Thus, the present invention makes possible a method of immunizing an individual comprising administering to an individual in need of

immunization an effective amount of a vaccine, in which the vaccine comprises a plurality of one or more distinct carbohydrate-based ligands and, optionally, one or more distinct non-carbohydrate-based ligands, which carbohydrate-based ligands at least are bound to and presented for multivalent interaction on a scaffold or on the surface of a solid support.

It is yet another aspect of the present invention to provide an assay method for a smaller collection of different types of carbohydrate ligands or for just a single type of carbohydrate ligand. Carbohydrate ligands having the same identity are preferably assayed using multiple copies of the same ligand presented on a solid support, such as a microtiter plate, a glass slide, or a solid or porous bead.

The present invention makes it possible for the first time to prepare rapidly a large number of carbohydrate derivatives by combinatorial synthetic methods, and to screen them rapidly for their binding affinity to proteins or other receptors of interest in a way that permits multivalent binding. The present invention makes it possible to discover novel ligands without resorting to traditional, labor-intensive organic synthesis and which ligands may have gone unrecognized by conventional assays, especially conventional, solution-based, affinity assays.

These and other objects of the invention should be apparent to one of ordinary skill in the art on consideration of the disclosure provided herewith.

4. Definitions

For the purposes of this disclosure, the following terms are defined as follows:

A *monosaccharide* refers to a pentose, hexose, heptose, or octose sugar, analog, or derivative

thereof, including, but not limited to, deoxy sugars, dideoxy sugars, amino sugars and sugar acids. These terms include the protected and unprotected forms thereof (that is, in which selected reactive groups, typically oxygen- or nitrogen-bearing groups, of the carbohydrate monomer or monosaccharide have been either temporarily blocked to prevent their undergoing a reaction under the conditions of a specific transformation or left exposed and available for possible participation in a reaction, respectively).

Thus, a *protecting group* is any chemical moiety that is temporarily attached to a reactive functional group of a given molecule to mask the functional group's reactivity while chemical reactions are permitted to proceed elsewhere on the molecule. Protecting groups preferred for protecting the reactive functional groups of sugars include, but are not limited to, alkyl, benzyl, acyl and silyl protecting groups. Many other s are well known to those of ordinary skill.

A *carbohydrate monomer* is a type of monosaccharide which is capable of influencing the stereochemical course of a glycosylation reaction such that the resulting glycosylation product bears substantially the stereochemistry desired (e.g., a 1,2-*cis* relationship among the substituents on the 1- and 2-positions of the glycosidic ring). A carbohydrate monomer is a particular type of *glycosyl donor*, as defined below.

A *carbohydrate, disaccharide, oligosaccharide,* or *polysaccharide* each refers to a molecule or a portion thereof, which is comprised of two or more monosaccharides that are joined by a glycosidic linkage. A *sugar* is any carbohydrate, disaccharide, oligosaccharide, polysaccharide, or monosaccharide.

The term *carbohydrate-based ligand* refers to a substance having an affinity for a given receptor,

such as a carbohydrate-binding protein, enzyme, nucleic acid, lipid and the like, and is composed solely or partially of carbohydrate moieties. The term *carbohydrate ligand* may be used interchangeably with *carbohydrate-based ligand* in this disclosure.

A *low-affinity* ligand is one that, in solution, binds to a receptor with a dissociation constant of from about one hundred micromolar to about one hundred millimolar.

A *glycoconjugate* refers to any molecule, substance, or substrate, including a solid, that includes a monosaccharide, carbohydrate, disaccharide, oligosaccharide, or polysaccharide covalently attached or adhered to a non-sugar chemical, biochemical, biological, or inorganic moiety. Preferred glycoconjugates include, but are not limited to, small molecules conjugated to the sugar (e.g., heteropolyaromatic-sugar conjugates, nucleosides, nucleoside analogs and the like), glycopeptide, glycoproteins, glycolipids and the like.

A *glycosyl donor* is a sugar with a leaving group (or potential leaving group) on at least one of its anomeric carbon which, under appropriate conditions, is capable of participating in a glycosylation reaction by which such anomeric carbon becomes covalently attached to a second moiety, typically a *glycosyl acceptor*, as defined below, or a nucleophile.

A *glycosyl acceptor* is any moiety, including a sugar, having the capacity to participate as the second moiety in the above-mentioned glycosylation reaction by virtue of a nucleophilic (or potentially nucleophilic) group present among the groups or substituents of such moiety, such that a covalent bond is formed between the anomeric carbon of such glycosyl donor and such nucleophilic (or potentially nucleophilic) group.

The phrase "sulfoxide-mediated glycosylation reaction" refers to the glycosylation technique first described by Kahne *et al.* in *J. Am. Chem. Soc.* (1989) 111:6881.

The terms "lower alkyl," "lower alkoxy," "lower acyloxy," or "lower alkenyl" refer to such substituents, as the case may be, having one to five carbon atoms, in either a linear or branched arrangement. The term "aryl" refers to aromatic groups, such as phenyl rings, naphthyl rings and the like, optionally bearing one or more substituents on the various ring positions. The term "heterocycle" refers to five-membered or six-membered aromatic rings containing one or more nitrogen, oxygen, or sulfur atoms, optionally bearing one or more substituents on the various ring positions. Such optional "substituents" may be any substituent that is chemically compatible with the aryl or heterocyclic ring and with the overall molecule of which such aryl or heterocyclic ring may be a part. Various aryl and heterocyclic rings may also be referred to herein as "substituted or unsubstituted" phenyl, naphthyl and the like to designate whether or not an optional substituent is present, respectively.

A *multivalent support* is any material or macromolecule to which more than one carbohydrate ligand can be attached, and includes, but is not limited to, organic dendrimers and polymers, glasses, metals and metal oxides, in any physical form such as solutions, emulsions, suspensions, beads, fibers, or planar surfaces. The term *solid support* or *solid phase* means a solid or porous material that is substantially insoluble in typical aqueous or non-aqueous solvents. Despite being insoluble, such supports may swell, however. Most solid supports or solid phases generally make adequate multivalent supports.

The *detection* of a substance refers to the

qualitative determination that the substance is present and may also refer to the quantitative measurement of the amount of the substance present. Detection may be made by any means, including but not limited to affinity-based, physical, optical, radiometric, photometric, electrochemical, or spectroscopic methods. All such detection methods are intended to be within the scope of the present invention. There are a variety of detection methods known in the art, and it is well within the capacity of the skilled practitioner to choose the method most appropriate or convenient for each situation.

The *selection* of a particular ligand-probe interaction refers to the process of selectively recognizing the interaction of interest from among a potentially large number of possible interactions. The selection step includes, but is not limited to, the detection of the desired interaction. For instance, selection may include the selective resolution of one or a few beads from a vessel filled with different beads or one or two wells from a multi-well plate.

A *detectable moiety* is any particle, molecule, fragment of a molecule, or atom whose presence and concentration can be readily measured, preferably by automated analytical instruments. The detectable moieties include, but are not limited to, radioactive isotopes, fluorescent molecules, chemiluminescent compounds, chromophores, high-affinity ligands or antigens, haptens, colloidal metal, enzymes, or other species or catalysts that can either produce or be manipulated to provide detectable products.

An *immune system response* includes any response by the body to invasion by a pathogen or to a cell affected by a disease caused by a pathogen, such as bacterial or viral infections, or caused by a hyperproliferative condition, such as neoplasticity, tumor growth, cancer, metastasis and the like and

further includes any humoral or cell-mediated immune response.

5. Brief Description Of The Figures

Fig. 1 illustrates the synthetic scheme for the preparation of compounds 1-20.

Fig. 2 illustrates the synthetic scheme for the preparation of compounds 21-D-26.

Fig. 3 illustrates the synthetic scheme for the preparation of compounds 27-33.

Fig. 4 illustrates the synthetic scheme for the preparation of compounds 34-L-45.

Fig. 5 illustrates the synthetic scheme for the preparation of compounds 46-53.

Fig. 6 illustrates the synthetic scheme for the preparation of compounds 54-66.

Fig. 7 illustrates the synthetic scheme for the preparation of compounds 67-77.

Fig. 8 illustrates the synthetic scheme for the preparation of compounds 78-88.

Fig. 9 illustrates the synthetic scheme for the preparation of compounds 81-95.

Fig. 10 illustrates schematically the synthesis of the disaccharide residue Galβ(1-3)GalNAc-β-thiophenyl ether on a solid phase support. Other sugar residues are prepared similarly using different precursor materials, glycosyl donors and/or reagents. For example, the disaccharide residue Galβ(1-3)GlcNAc-β-thiophenyl ether is prepared using the GlcNAc-β-thiophenyl ether precursor illustrated at the bottom of the Figure.

Fig. 11 shows the results of an aggregation study of TentaGel beads. The right panel shows that underivatized beads do not aggregate when treated with *Arachis Hypogaea* lectin at 25 μg/mL. The left panel shows how derivatized beads aggregate under the same conditions.

Fig. 12 illustrates the results of a

colorimetric assay of a four-carbohydrate mixture (magnified 100-fold), along with the structures of the four resin-bound carbohydrates.

Fig. 12A tabulates the results of the screening of the four-carbohydrate library. The "a" series use R = $OCH_2C(O)NH$-TentaGel; "b" series use R = H; "c" series use R = $OCH_2C(O)NHCH_2CH_2OCH_3$.

Fig. 13 diagrams the steps of a particular colorimetric version of the assay of the invention. The colorimetric assay is used for the selection of a specific disaccharide-lectin interaction among those possible with the various members of the library. The multivalent nature (and, perhaps, other features) of the interaction is not illustrated in this Figure.

Fig. 14 shows the precursors (or monomers), glycosyl donors and additional reagents used in a particular embodiment of the present library. A library of approximately 1500 distinct carbohydrate-based ligands is prepared by a "split and mix" method described herein. The stereochemical configuration of the putative natural substrate of the lectin used in the subsequent assay is also illustrated.

Fig. 15 is a reproduction of a photograph showing the colored bead selected by the assay of the invention among the large population of beads contained in the assay vessel. A number of different carbohydrate-based ligands, particularly unnatural ones, are identified by the methods of the invention.

6. Detailed Description Of Preferred Embodiments

The present invention, in general terms, provides a method for discovering or identifying ligands for receptors. The present invention is particularly useful for discovering or identifying ligands for receptors which utilize polyvalent interactions with their ligands. More particularly, the present invention provides a method for identifying or discovering carbohydrate-based ligands

for carbohydrate-binding receptors, including peptides or proteins, especially those carbohydrates-based ligands that in solution exhibit a low affinity for the carbohydrate-binding receptor. The methods disclosed are especially suitable for screening libraries of compounds but may also be applied to the study of smaller collections of carbohydrate-based ligands or even single ligands on an individual basis.

The invention involves the presentation of multiple copies of a ligand, attached to a solid support, to a receptor, in order to take advantage of any polyvalent interactions that the receptor might be capable of. A receptor-ligand interaction is then detected and/or selected from which information about the ligand can be obtained. Preferably, the selection/detection methods and identification schemes can be carried out using conventional methods, such as known affinity-binding detection methods, "sequencing" techniques, microanalytical techniques and spectroscopic methods.

In a preferred embodiment of the invention, single or distinct resin-bound carbohydrate ligands and, subsequently, a spatially resolved carbohydrate library, are synthesized on polymer beads as a solid support.

For the creation of the library, a "split and mix" combinatorial approach is employed, although "parallel" approaches can also be used. Further, the individual beads can be encoded using available chemical tagging technologies. As one of ordinary skill can appreciate, chemical tagging techniques facilitate the rapid structural identification of the ligand attached to or derived from a particular bead. See, for example, Barchart, A. and Still, W. C., in *J. Am. Chem. Soc.* (1994) 116:373-374; Nestler, P., et al., in *J. Org. Chem.* (1994) 59:4723-4724; Ohlmeyer, M. H. J. et al., in *Proc. Natl. Acad. Sci. USA* (1993)

90:10922-10926; Baldwin, J. J. et al., in *J. Am. Chem. Soc.* (1995) 117:5588-5589; International Publication Number WO 94/08051. While the use of tagging technology lends convenience to the structural elucidation of novel ligands, the preparation and selection of the ligand does not depend on any tagging, sequencing, or affinity binding technique. Structural analysis can also be performed directly by using traditional means, including, but not limited to, analytical, elemental and spectroscopic methods.

Still other known methods of identifying library members, which are detected by an appropriate assay, include library deconvolution/resynthesis techniques and spatial addressing. Moreover, smaller libraries can also be prepared by parallel synthesis, obviating a decoding step.

Also, while a number of synthetic methods can be used in the present invention, the preferred method for forming the library of the invention comprises the use of a sulfoxide-mediated glycosylation reaction step, i.e., one that leads to the formation of new glycosyl bonds.

In an example for the synthesis of a single carbohydrate, the disaccharide β-Gal-((1-3))-GalNAc, a known ligand for peanut lectin, is prepared. Peanut lectin is representative of any carbohydrate-binding protein that utilizes a multivalent mode of carbohydrate recognition. Polymer beads having multiple copies of this disaccharide attached to its surface agglutinate upon exposure to peanut lectin. In contrast, a similar exposure to peanut lectin of identical beads not bearing the disaccharide failed to result in agglutination. The results of this agglutination study are shown in Fig. 11.

The agglutination assay described above demonstrates that a carbohydrate binding protein containing multiple carbohydrate binding sites causes

agglutination of the carbohydrate-derivatized polymer beads. The experiment thus shows the availability of the support-bound disaccharide for polyvalent receptor binding. The experiment further demonstrates the usefulness of the invention for detecting qualitatively the binding activity of individually synthesized carbohydrates on a solid support. Moreover, the concentration of lectin required for agglutination of the beads can be used to quantitate the binding activity of the ligand. However, the agglutination assay cannot be used to select resin-bound carbohydrates that bind to a receptor from a mixture of resin-bound carbohydrates.

In order to identify resin-bound carbohydrates that bind to a particular receptor from a library of resin-bound carbohydrates, the colorimetric assay described in Example 7.35 is used. To demonstrate the utility of the assay for differentiating between closely related resin-bound carbohydrate ligands in a mixture of resin-bound carbohydrates ligands, the disaccharides shown in Fig. 12 are synthesized separately on TentaGel S RAM resin. These disaccharides differ only in the stereochemistry at C4 and at the anomeric linkage of the first sugar. Equal portions of each resin are combined and assayed as described in 7.35. Approximately 25% of the beads stain dark purple, 25% stain light purple, and 50% of the beads remain white (Fig. 12). The beads are separated according to color and the products were removed from the beads by hydrolysis with TFA and identified by correlation with authentic standards. The dark purple-staining beads are found to contain Galβ(1-3)GalNAc-β-thiophenyl ether (Fig. 12A). The light purple beads are found to contain Galβ(1-3)GlcNAc-β-thiophenyl ether and Galβ(1-3)GalNAc-α-thiophenyl ether. The unstained beads are found to contain Galβ(1-3)GlcNAc-α-thiophenyl ether.

This experiment demonstrates that the

colorimetric assay described in Example 7.35 can be used to differentiate closely related carbohydrate ligands. Although the carbohydrate ligands have similar affinities for the *Bauhunia purpurea* lectin in solution as evaluated by an assay which measures inhibition of agglutination of erythrocytes (Laboratory techniques in biochemistry and molecular biology: Glycoprotein and proteoglycan techniques, Beeley, J.G. p. 327-333, Elsevier Science: Amsterdam 1985), the avidities of the carbohydrate-derivatized beads are sufficiently different that the individual carbohydrates can easily be differentiated.

In another embodiment of the invention, a library of 1269 resin-bound carbohydrates is synthesized on polymer beads. Although the library described herein is synthesized on TentaGel polymer beads, the present invention is not limited to TentaGel or even to a polymer bead. For instance, rather than being on polymer beads, the library members can be arrayed on a planar support, such as a microtiter plate or glass slide.

In another embodiment, the library members can be attached to a functionalized surface, such as a modified polyethylene substrate.

The present invention is intended to include within its scope the use of any modified solid support that allows the bound carbohydrate-based ligands enough geometric flexibility to achieve polyvalent binding to a receptor, i.e., which provides a multivalent support. In particular, the presentation of "polydentate" carbohydrate-based ligands permits the practitioner to (1) assay receptor binding to the members of the library, and (2) associate any observed binding with a particular member of the library or subset thereof.

The active members of the library can be identified by a variety of methods known in the art. For example, if synthesized in a spatial array - the

members of the library could be identified by physical location on the array. By using a split and mix strategy using polymer beads, members of the library could be identified using an associated chemical or physical tag, or by direct structure determination, or by a deconvolution strategy, or by a combination of deconvolution and resynthesis.

The method of the present invention can be used to generate and screen carbohydrate-based ligands for potentially any biological receptor. Also, the present method is especially valuable for discovering new, clinically useful compounds that exhibit an affinity for carbohydrate-binding receptors, such as cell adhesion molecules, for example. Such newly discovered compounds have the potential to exhibit useful biological activity, including, but not limited to, agonist, antagonist, inhibitory, augmenting, or simply activity that interferes or disrupts inter or intracellular signal transduction.

The carbohydrate library is preferably constructed on a commercially available synthesis resin, a polyether chain-modified polystyrene sold under the trade name TENTAGEL, using a "split and mix" synthesis approach. The resin is composed of a cross-linked polystyrene base to which poly(ethylene glycol) chains are attached. The library members are attached to the poly(ethylene glycol) chains. Using six separate reactor vessels, six different monomers are attached to the resin via a thioether linkage. The resins are mixed and then split into twelve equal batches, each of which is glycosylated with one of twelve different glycosyl donors to produce seventy-two different di- or trisaccharides. Because it is desirable that a library constructed using a split and mix synthesis strategy have a single product on each bead, the glycosylation method used should preferably achieve glycosylation stereospecifically for all the different donor/acceptor pairs in all the

reactor vessels. Accordingly, the preferred method of glycosylation is the sulfoxide-mediated glycosylation reaction.

Following the glycosylation reactions, the resulting batches are recombined, mixed and split into twenty separate batches. Nineteen of these batches are treated with trimethylphosphine to reduce the azides to amines. Eighteen of these nineteen trimethylphosphine-treated batches are acylated. The twenty batches of resin are recombined. All hydroxyl protecting groups are removed to produce a library containing approximately thirteen hundred carbohydrates.

Most carbohydrate-binding proteins recognize the non-reducing end of their carbohydrate ligand. Therefore, the carbohydrates in the exemplified embodiment of the invention are deliberately synthesized with the reducing end directed toward the solid surface, with subsequent sugar or other reaction units added with the non-reducing end directed outward. This orientation facilitates library screening while the carbohydrates are still attached to the support.

The synthesis of carbohydrate libraries from the non-reducing toward the reducing end would present substantially the same library in a different orientation. Accordingly, such a reverse orientation is contemplated and is intended to be within the scope of the present invention.

The exemplified library is designed to include the natural ligand for peanut agglutinin, a carbohydrate-binding protein that agglutinates neuraminidase-treated erythrocytes. As stated above, this protein system is a good model system for many carbohydrate-binding protein-carbohydrate ligand interactions because the affinity of peanut agglutinin for its natural ligand is low. What is more, it utilizes a polyvalent strategy to achieve

activity (agglutination).

Referring now to Fig. 13, to screen the library, 10 mg of resin containing approximately 10,000 beads (approximately six copies of the library) is treated with biotinylated peanut agglutinin. The beads are washed and treated with streptavidin conjugated to alkaline phosphatase. After a brief incubation and washing step, the enzyme substrate is added. Beads containing bound lectin change color as the alkaline phosphatase converts the soluble substrate to an insoluble dye that precipitates within and on the surface of the bead. Different beads change color at different rates, reflecting different concentrations of bound lectin. Colored beads are selected at different time intervals after the addition of the soluble dye.

In fact, 8 beads out of the thousands of beads are selected within the first five minutes of the addition of dye. (See, Fig. 15.) Thus, the selection process appears to be amplified in the solid phase and demonstrates that the assay of the invention is exquisitely both selective and sensitive. Contrary to what may have been expected based on the relatively similar binding characteristics of the ligands in solution, beads bearing ligands having a stereochemical configuration different from the putative natural ligand are singled out by the present method. Hence, the multivalent presentation of the ligands appears to have a profound effect on the relative affinities of the various ligand configurations for the lectin probe.

Despite the individual carbohydrates in the library having low affinities when in solution, with binding constants in the millimolar to micromolar range, and despite the minimal differences between these ligands, the results of the assay confirm the unparalleled capacity of the present invention to

distinguish between carbohydrate-based ligands of very similar binding affinities.

6.1. General Experimental Methods

6.1.1 Materials: TentaGel resin is purchased from RAPP Polymere (TentaGel S NH_2, 130 μm, 0.3 mmol g^{-1} capacity). Dichloromethane (CH_2Cl_2), *N,N*-dimethylformamide (DMF), tetrahydrofuran (THF), diisopropylethylamine (DIEA) and trifluoromethanesulfonic acid (TFA), and bovine serum albumin (BSA) are from Aldrich. 1-methyl-2-pyrrolidinone (NMP), 1-hydroxybenzotriazole (HOBt) and 2-(1H-benzotriazol-1-yl)-1,1,3,3,-tetramethyluronium hexafluorophosphate (HBTU) are from Applied Biosystems. Lyophilized powders of lectins and alkaline phosphatase-conjugated streptavidin, as well as solutions of 5-bromo-4-chloro-3-indolyl phosphate (BCIP)/nitroblue tetrazolium (NBT) liquid substrate system and *p*-nitrophenyl phosphate (pNPP) are from Sigma. Rabbit blood is purchased from Remel. Phosphate buffered saline (PBS) is 150 mM NaCl, 7.3 mM Na_2HPO_4, and 2.7 mM NaH_2PO_4, adjusted to pH 7.2. PBST is 0.05% (v/v) Tween-20 in PBS. Tris buffered saline (TBS) is 500 mM NaCl and 20 mM Tris, adjusted with dilute HCl to pH 7.5. TBST is 0.05% (v/v) Tween-20 in TBS. Alkaline phosphatase buffer (AP) is 100 mM NaCl, 5 mM $MgCl_2$, and 100 mM Tris, adjusted to pH 9.5.

6.1.2. General Procedure for the Synthesis of Resin-Bound Disaccharides: TentaGel resin (0.674 g) is suspended in 15 mL of NMP, and to this is added 4'-(carbonylic acid)methyleneoxyphenyl 3-*O*-acetyl-2-azido-4,6-*O*-

benzylidine-2-deoxy-1-thio-α-D-galactopyranoside (0.122 g, 0.243 mmol), DIEA (0.22 g, 1.3 mmol), and HOBt/HBTU solution (0.45 M in DMF, 2.2 g, 0.93 mmol). The suspension is shaken for 12 hours, and the resin is washed with CH_2Cl_2, NMP and DMF. A solution of anhydrous hydrazine in DMF (1:7, 16 mL) is added, and the reaction mixture is shaken for 9 hours until acetate hydrolysis is shown to be complete by infrared analysis (potassium bromide pellet). The resin is washed with DMF, H_2O, methanol and CH_2Cl_2. Optionally, the resin is encoded for the glycosyl acceptor by known tagging methodologies. A resin portion (0.100 g) is suspended in 5 mL of CH_2Cl_2, agitated with argon for 10 min. Phenyl 2,3,4,6-tetra-*O*-pivaloyl-1-thio-β-D-galactopyranoside sulfoxide (0.24 g, 0.40 mmol) and 2,6-di-*tert*-butyl-4-methylpyridine (0.13 g, 0.65 mmol) are dissolved in anhydrous CH_2Cl_2 (5 mL) and added to the resin.

The suspension is cooled to -60 °C and a solution of trifluoromethanesulfonic anhydride (34 μL, 0.20 mmol) in 1 mL of CH_2Cl_2 is added. After warming to 0 °C over 1-2 hours, the resin is washed with saturated sodium bicarbonate, H_2O, methanol, diethyl ether, CH_2Cl_2 and toluene. The resin is dried and re-subjected to the glycosylation conditions. The resin is optionally encoded, suspended in thiolacetic acid (25 mL), shaken at room temperature for 27 hours, washed with CH_2Cl_2 and dried. The resin is suspended in 20% tetrahydrofuran (THF)/CH_2Cl_2 (20 mL), shaken at room temperature for 30 min and washed with CH_2Cl_2. The resin is swelled in a solution of 20% tetrahydrofuran (THF)/methanol (20 mL) for 10 min and ground lithium hydroxide monohydrate (0.20 g, 4.8 mmol) is added. The reaction mixture is

shaken at room temperature for 11 hours, and washed with H_2O until the pH of the filtrate is determined to be neutral. The resin is then dried *in vacuo* for 12 hours.

6.1.3. **Aggregation Study:** 1 mg samples of TentaGel beads derivatized with Galβ(1-3)GalNAc-β-thiophenyl glycoside and underivatized beads are placed in separate wells of a 96 well microtiter plate and swollen in PBS buffer. The buffer is removed, and 100 µL of *Arachis Hypogaea* lectin (10-1000 µg/mL in PBS buffer) is added to each well. The beads are examined under a microscope after 1-2 hours.

6.1.4. **Hemagglutination Assay (HA):** A stock solution of the lectin is made by dissolving 5 mg of *Bauhinia Purpurea* lectin in 2.5 mL of PBS. Serial dilutions of lectin in PBS are prepared, and 50 µL of each solution is transferred into 12 microtiter plate wells. 50 µL of a 2% suspension of rabbit erythrocytes in PBS is added to each well and incubated at room temperature on an orbital shaker for 1 hour. Agglutinated cells form a carpet covering the bottom of the well; non-agglutinated cells form a compact button at the center of the well. The titer is defined as the last dilution well before the erythrocyte button begins to form. The HA titer for *Bauhinia Purpurea* lectin is ca. 1 µg/mL.

6.1.5. **Hemagglutination Inhibition Assay (HAI):** Stock solutions of **1b-4b** (5 mg/mL) (See, Fig. 12A) in PBS are prepared and serial dilutions are made. 25 µL of each sugar solution is added to single wells of a microtitre plate that contain 25 µL of a 16 µg/mL lectin solution

in PBS and incubated at room temperature on an orbital shaker for 1 hour. To each well is added 50 µL of a 2% suspension of erythrocytes. The plate is gently agitated for 10 min and then incubated at room temperature for 1 hour. The final lectin concentration is 4 µg/mL, which is 4x the dose of the HA titer. The end point is defined as the lowest sugar concentration which inhibits agglutination.

6.1.6. Colorimetric Assay for Four Carbohydrate Mixture: A portion of resin, which contains equal portions of **1a-4a** (10 mg total), is washed with PBST buffer (3 x 1 mL, 10 min). The beads are incubated for 30 min at room temperature in 1 mL of PBST containing 3% BSA and washed with PBST (3 x 1 mL, 5 min) containing 1% BSA. The beads are incubated in 1 mL of *Bauhinia Purpurea* lectin (0.1 µg/mL in PBST containing 1% BSA) at room temperature for 3 hours and then washed with TBST buffer (3 x 1 mL, 5 min) containing 1% BSA. The resin is incubated for 20 min at room temperature in 1 mL of alkaline phosphatase-conjugated streptavidin (10 µg/mL in TBST containing 1% BSA) and then washed with alkaline phosphatase buffer (3 x 1 mL, 5 min). A small portion of the resin (~1/3) is stained with 200 µL of BCIP/NBT. Staining is terminated after 30 min by washing the beads twice with 200 µL of 20 mM sodium ethyleneaminetetracetic acid, pH 7.4. The dark purple, light purple and colorless beads are pulled out for analysis, including decoding if encoded, using 50 µL micropipettes.

6.1.7. Colorimetric Assay for Carbohydrat Library: Screening of the larger carbohydrate library follows the same procedure as

that provided above, except that the lectin concentration is 10 μg/mL for the larger library.

6.1.8. Inhibition Assay: TentaGel resin (1.0 mg) derivatized with Galβ(1-3)GalNAc-β-thiophenyl ether (**1a**) is added to each well of a 96-well MultiScreen Filtration Plate (Millipore). Each portion of resin is washed with PBST buffer (3 x 100 μL, 5 min). The buffer solution is removed from each well simultaneously by placing the filtration plate on a MultiScreen Vacuum Manifold (Millipore). Each portion of resin is incubated for 30 min with 100 μL of PBST containing 3% BSA and washed with PBST containing 1% BSA (3 x 100 μL, 5 min). Sugar solutions of 10 different concentrations are prepared from sugar stock solutions (5 mg/mL in PBST containing 1% BSA). A solution of *Bauhinia Purpurea* lectin (1 mg/mL in PBS) is added to each well to afford a final lectin concentration of 100 μg/mL. The combined lectin/sugar solutions are incubated at room temperature for 1 hour, and 100 μL of each solution is added to the resin. The plate is agitated on an orbital shaker at room temperature for 3 hours. The resin is washed with TBST containing 1% BSA (3 x 100 μL, 5 min).

A solution (100 μL) of alkaline phosphatase-conjugated streptavidin (10 μg/mL in TBST containing 1% BSA) is added to each well, and the plate is incubated at room temperature for 20 min. The beads are washed with alkaline phosphatase buffer (3 x 100 μL, 5 min) and transferred into a 96-well flat-bottomed microtiter plate. A pNPP solution (100 μL) is added to each well using a 12-channel pipetman, and the color development at 405 nm is monitored using a microplate reader.

6.2. Discussion

Following the methods of the present invention, large numbers of polyvalent carbohydrate ligands are produced simultaneously in a format that permits parallel screening. By synthesizing carbohydrates directly on a solid support and screening them for binding, the invention is able to exploit some of the advantages of combinatorial chemistry in the course of studying carbohydrate recognition processes (vide infra).

6.2.1. Selecting the Solid Support.

At least two criteria are important in choosing a solid support: ease of synthesis and ease of screening. In the present study, the disaccharide Galβ(1-3)GalNAc-β-thiophenyl ether **(1a)** is constructed on TentaGel resin. The sulfoxide glycosylation reaction proceeds stereospecifically and in near quantitative yield. Subsequent chemical transformations also worked well on the resin.

The synthesis is carried preferably out from the reducing to the non-reducing end of the ligand so that the carbohydrates are presented in a way that mimics cell surface carbohydrates. This orientation also permits direct screening of the derivatized beads for binding. To evaluate further the suitability of TentaGel resin for on-bead screening, samples of the Galβ(1-3)GalNAc beads and underivatized beads are treated separately with varying concentrations of *Arachis Hypogaea* (peanut) lectin, a protein known to recognize Galβ(1-3)GalNAc.

As shown in Fig. 12 (right panel), the underivatized beads do not aggregate at lectin concentrations ranging from 10-1000 μg/mL. In contrast, the carbohydrate-derivatized beads (left

panel) aggregate at a lectin concentration of 25 μg/mL. *Arachis Hypogaea* lectin also causes erythrocytes to aggregate - or agglutinate - in this concentration range in a process believed to involve polyvalent interactions between the lectin and carbohydrates on the surfaces of different cells. Thus, the data suggest that the derivatized beads aggregate due to multivalent interactions between the lectin and carbohydrate ligands on different beads.

Consistent with this hypothesis, the aggregation can be prevented by increasing the protein concentration such that the protein coats the entire surface of each bead and makes multivalent interactions involving carbohydrates on different beads impossible.

Taken together, these results suggest that the protein is involved in polyvalent interactions with the carbohydrate derivatized beads.

6.2.2. Development of a Colorimetric Assay for Detecting Binding. By the methods of the present invention, an assay is provided which can discriminate between different carbohydrate-derivatized beads.

Accordingly, four similar carbohydrate ligands (**1a-4a**) are synthesized on TentaGel resin (Fig. 12A). Galβ(1-3)GalNAc (**1a**) is a known ligand for *Bauhinia purpurea* lectin. The structures of the three other carbohydrates differ from **1a** in terms of the stereochemistry at the C4 position and/or at the anomeric position of the sugar directly attached to the resin. The C4 stereochemistry is varied because solution binding studies have shown that the lectin is sensitive to the stereochemistry at this position and binds the C-4 equatorial isomer with a three-fold lower affinity relative to the axial isomer. The

configuration of the internal glycosidic linkage is varied to probe the effect of ligand presentation on binding.

The beads can be encoded during the synthesis, if desired, using known technology (vide supra), so that the four carbohydrates can be screened in parallel, and the results assessed more quickly. To screen the beads, a sample of resin containing equal amounts of the four different carbohydrate-derivatized beads is incubated with biotinylated *Bauhinia purpurea* lectin (0.1 μg/mL), followed by streptavidin-linked alkaline phosphatase. The beads are then stained with BCIP/NBT (5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium), which is converted by alkaline phosphatase to an insoluble purple polymer which precipitates on the surface of the beads. Other methods of detection can be used according to the particular requirements or preferences of a given library and screen.

Beads that stain rapidly are presumed to have more enzyme-linked conjugate, and hence more bound lectin, than the other beads. The results of this colorimetric assay show that approximately 25% of the beads stain very darkly, 25% of the beads stain lightly, and 50% of the beads do not stain within the time frame of the assay (Fig. 12). Twenty dark purple beads, twenty light purple beads, and eighteen unstained beads are removed from the mixture. It is determined that all 20 dark purple beads contain Galβ(1-3)GalNAc-β-thiophenyl (**1a**), but none of the light purple or unstained beads contain ligand **1a**.

Thus, the assay of the present invention clearly distinguishes the best ligand from three other closely related ligands. It is also apparent from the assay that the worst polyvalent ligand is **4a**, in which both the C4 and anomeric

stereochemistry differ from the known ligand, **1a**.

Ligands **2a** and **3a** have similar avidities, although the ratio of stained to unstained beads suggests that **2b** is a better ligand. Hence, the two apparently best ligands contain the β-stereochemistry at the internal glycosidic linkage.

To evaluate the relationship between the polyvalent avidities and monovalent affinities of the carbohydrate ligands, the thiophenyl glycosides **1b-4b** are each synthesized and evaluated separately for their relative *solution* affinities for *Bauhinia purpurea* lectin using a standard hemagglutination inhibition assay. The results from this solution assay show that **1b**, inhibits agglutination at concentrations four-fold to eight-fold *lower* than the other three thiophenyl glycosides. *There is essentially no difference in the solution affinities of the other three carbohydrates.* Furthermore, changing from the thiophenyl glycosides to a set of thiophenyl derivatives containing an acetamide ethanolamine chain (**1c-4c**), which chain resembles the linker to the resin, has no effect on the relative solution affinities. Hence, the acetamide ethanolamine chain does not appear to interact with the protein.

The results suggest that there may not be a good correlation between solution affinities and on-bead avidities. The on-bead screen shows detectable differences between the polyvalent avidities of the four carbohydrate ligands. In contrast, the agglutination inhibition assay shows that only one of the four carbohydrates has a measurably higher binding affinity. Although the best inhibitor in solution proved to be the best polyvalent carbohydrate ligand, work on the alrger

library disclosed herein suggests that this finding is a coincidence of the small number of compounds examined (vide infra).

6.2.3. Screening of a Carbohydrate Library. An encoded library designed to contain ~1300 different compounds with seventy-two different glycosidic linkages is synthesized. Using an assay procedure similar to that described above for the four compound mixture, 10 mg of the larger resin-bound library, or approximately six copies of each carbohydrate, is screened against *Bauhinia purpurea* lectin at a concentration of 10 μg/mL. Fewer than 0.3% of the beads in a pool of ~9,000 beads show a significant amount of staining. Twenty-five dark purple beads are selected from the library over a period of twenty minutes.

Five copies each of two closely related compounds having stereochemical configurations different from the putative natural ligand are identified among the twenty-five beads. Both compounds contain the same disaccharide structure substituted with two different hydrophobic N-acyl groups. Three other beads are found to contain the same disaccharide structure as that found above with different, but also hydrophobic, N-acyl groups than the two N-acyl groups found above.

Hence, over half of the stained beads have the same core disaccharide, suggesting a remarkable degree of specificity in the binding assay. It should be noted that of the remaining twelve stained beads, none appear more than once and no pattern is evident. We consider these beads, which take longer on average to stain than the more avid ligands, to represent the noise in the enzyme-linked assay.

The most avid disaccharide contains an α

glycosidic linkage between the two sugars while the known ligand for this lectin contains a β linkage. In addition, the preferred ligands have an equatorial hydroxyl group at the C4 position of the resin-linked sugar even though previous results suggest that the axial hydroxyl group is preferred. Finally, the preferred ligands have an axial anomeric linkage to the resin.

The screen of the four compound mixture suggests that the equatorial thiophenyl linkage to the resin is preferred over the axial stereochemistry, at least when the glycosidic linkage between the two sugars is equatorial.

Although both the α- and β-thiophenyl derivatives of the known ligand, Galβ(1-3)GalNAc, are included in the library, neither appear among the pool of stained beads. Thus, the ligands identified from the library bind the lectin more avidly than the known ligand, despite containing a number of structural changes which are individually unfavorable.

In the course of evaluating the relationship between monovalent binding affinities in solution and polyvalent avidities, it is found that the known ligand **1c** inhibits binding at a concentration of 25 μM and is considered to be the best monovalent ligand *in solution*. The two ligands identified by the assay, in contrast, only inhibit lectin binding at concentrations of 35 and 46 μM, respectively. It is, therefore, apparent that the solution affinities of monovalent ligands do not correlate well with the corresponding polyvalent avidities. The presentation of the carbohydrates on the polymer beads clearly has a profound influence on their avidities. The way in which carbohydrates are presented on cell surfaces undoubtedly also has a significant influence on their receptor-binding interactions. Therefore,

caution must be exercised in drawing conclusions about the structure-function relationships of surface-bound carbohydrates from solution affinities.

Hence, in a particular aspect of the present invention, it is shown that carbohydrate-derivatized beads resemble carbohydrate presenting cell surfaces in key respects. The carbohydrate-derivatized beads can be recognized by carbohydrate-binding proteins, and at low protein concentrations the recognition process involves polyvalent interactions. It is, therefore, believed that at least some of the conclusions drawn about the recognition of these carbohydrate-derivatized beads can also be applied to understanding carbohydrate recognition *in vivo*. One of the conclusions drawn is that the presentation of the carbohydrate ligand on the surface plays a critical role in determining how the carbohydrate ligand interacts with its receptor. Hence, there may be critical concentrations of a given carbohydrate ligand on the surface of a cell which determine the activation of inter- or intracellular communications initiated by interaction of the cell surface with a carbohydrate binding partner.

It cannot be overemphasized that the results of the parallel screen disclosed above show that polyvalent carbohydrate ligands can function with exquisite specificity. Two almost identical carbohydrate ligands are identified as specific ligands for a carbohydrate-binding protein in the presence of a collection of 1300 other carbohydrate ligands - many of which bind with similar or better affinity to the same protein when evaluated as monomers in solution. Thus, although protein-protein interactions may indeed play important roles in signalling pathways *in*

vivo, there is no need to invoke protein-protein contacts to explain the specificity of an event, since it could just as well be determined by a specific carbohydrate-mediated adhesion process.

6.3. Additional Supporting Disclosure

According to the present invention a library comprising a collection of distinct carbohydrate-based ligands is provided. In the instant library, a plurality of each ligand is bound to and presented on the surface of a resolvable portion of a solid support to permit: (i) multivalent interactions of the plurality of ligands with one or more probes bearing a plurality of carbohydrate binding sites (that is, the probe has the capacity to bind in a polyvalent fashion), and (ii) selection of at least one particular ligand-probe interaction. In a specific embodiment of the invention, the library is prepared by a method comprising a glycosyl bond-forming step, preferably a sulfoxide-mediated glycosylation reaction and more preferably, conducted in a solid phase. It should also be pointed out that the glycosyl bond forming step leads to the formation of a C-, N-, O-, S-, or P-linked glycoside.

The solid support to which the ligand is bound may comprise any solid or porous material, including but not limited to a planar support, separate wells, a multi-well microtiter plate, or a three-dimensional, spherically shaped substrate. Preferably the solid support comprises a plurality of solid or porous beads. In a particular embodiment of the invention, the solid support is substantially insoluble in a variety of solvents, including aqueous and non-aqueous solvents. For example, the preferred solid or porous beads are insoluble in tetrahydrofuran, methanol, methylene chloride, N-methyl pyrrolidinone and dimethylformamide. Preferably, the solid support comprises a synthetic

polymer, such as polystyrene or a modified polystyrene. More preferably, the solid support comprises a polyether chain-modified polystyrene, most preferably, commercially available TentaGel resin. As stated above, the resolvable portion of the solid support may comprise a well of a planar support or a bead of a spherically shaped support.

The size of the beads can vary depending on the particular application. Generally, the beads may have a wide range of diameters. For example, the bead may have an average diameter ranging from about 50 nm to about 5 μm, about 50 nm to about 1 μm, about 50 nm to about 0.5 μm, about 50 nm to about 250 nm, about 50 nm to about 100 nm, or about 75 nm to about 200 nm. Still in other applications, the bead may have an average diameter that is less than or equal to about 0.5 μm, less than or equal to about 0.3 μm, less than or equal to about 0.2 μm, or less than or equal to about 0.1 μm.

Moreover, the solid support may comprise one or more detachable scaffolds on the surfaces of an insoluble solid or porous bead. In a particular embodiment, the solid support comprises comprise derivatized beads, each having one or more detachable scaffolds on the bead surfaces, a plurality of each ligand being bound to the one or more scaffolds. Indeed, the scaffold can bear a plurality of one or more distinct carbohydrate-based ligands and, optionally, one or more distinct non-carbohydrate-based ligands. The scaffold can then be detached from the solid support, if desired, prior to conducting an assay or using the derivatized scaffold, e.g., prior to administration to an individual for a therapeutic, diagnostic, or prophylactic application.

In fact, the present invention contemplates a composition for use as a vaccine comprising a plurality of one or more distinct carbohydrate-based

ligands and, optionally, one or more distinct non-carbohydrate-based ligands, which carbohydrate-based ligands at least are bound to and presented on the surface of a solid support to permit the multivalent interaction of the plurality of one or more distinct carbohydrate-based ligands with one or more receptors associated with an immune system response, such that an individual, to whom an effective amount of the composition has been administered, is able to mount an appropriate immune response against a given disease that is caused by a given pathogen or which is characterized by the expression of a given marker on the surface of a cell affected by the disease. The composition of the present invention may further comprise a pharmaceutically acceptable carrier and may further include any adjuvants appropriate for amplifying or enhancing the desired immune system response (that is, increase the immunogenicity of the composition).

According to the present composition, optional non-carbohydrate-based ligands are also contemplated, which optional ligands may be administered before, after, or along with the carbohydrate-based ligands. In particular, such optional ligands may also be presented on the surface of the same or separate solid support or scaffold as the carbohydrate-based ligand. Examples of such optional ligands include, but are not limited to, small molecules, drugs, peptides, glycopeptides, proteins, glycoproteins, nucleic acids (e.g., deoxyribonucleic acids or ribonucleic acids), lipids, glycolipids, or combinations or complexes thereof.

When meant for administration into and circulation within the vascular system, the solid support preferably comprises an insoluble solid or porous bead having an average diameter that permits the bead to move substantially freely in an individual's circulatory system. Suitable sizes are

enumerated above, but preferably sizes are selected which would minimize clogging of an individual's blood vessels and/or capillaries.

Hence, the present invention also contemplates a method of immunizing an individual comprising administering to an individual in need of immunization an effective amount of vaccine comprising a plurality of one or more distinct carbohydrate-based ligands and, optionally, one or more distinct non-carbohydrate-based ligands, which carbohydrate-based ligands at least are bound to and presented for multivalent interaction on a scaffold or on the surface of a solid support. An example of a suitable scaffold is a dendrimer, a glycosaminoglycan, a glycan, or a piece of an extracellular matrix.

The ligands may be bound to the solid support or scaffold in any number of ways, directly or indirectly through a linker moiety. Preferably, the ligands are bound to functional groups on the surface of the solid support or scaffold via linker groups. Any one of a great variety of bifunctional linker groups known to those of ordinary skill in the art can be used as the linker moiety.

As a probe, one may use a substance that comprises one or more receptors. Such receptors may further comprise a sequence of amino acids (e.g., peptides or proteins, including one or more protein subunits), a piece of DNA, or a piece of RNA. The receptor may also form part of an enzyme, such as a protease. The preferred receptors bear two or more (i.e., a plurality of, a multiplicity of, or the capacity to interact with more than one carbohydrate ligand) carbohydrate binding sites. The probe may comprise an intact cell or a portion thereof. The cell may further be a prokaryotic cell or a eukaryotic cell, a bacterial, yeast, fungal, mammalian, animal, human, plant, or insect cell.

Furthermore, the desired cell probe may be selected from among those involved in a cell-mediated immune response, including B lymphocytes, T lymphocytes, natural killer cells, or neutrophils. The cell may also be selected from among those involved in the production of antibodies. Preferred cells may be phagocytic cells, tumor cells, infected cells, diseased cells, or cells from malignant tissue. Additional cells can be selected from antigen presenting cells or cells involved in a cell adhesion process.

In a particular application of the present invention, a library of carbohydrate ligands can be synthesized on TentaGel beads and screened against neutrophils to select the multivalent carbohydrate ligands that bind the neutrophils best. The beads are then washed to remove unbound neutrophils. Bound neutrophils can be detected by treating the beads with nitroblue tetrazolium, which is converted to an insoluble colored product by oxidative enzymes in the neutrophils. The beads that change color are selected from the library and washed extensively to remove bound neutrophils. The identity of the carbohydrates on the beads can then be determined by decoding (if the library is encoded) or by a deconvolution strategy involving resynthesis, or by some combination of mass spec of the hydrolyzed products and a deconvolution strategy.

Once the carbohydrate ligands that bind neutrophils well are determined, the plasma membrane proteins of the neutrophils can be solubilized and then passed down a column containing beads derivatized with the appropriate carbohydrate. The protein receptors on the plasma membrane that bind the multivalent carbohydrate ligands adhere to the column. The other membrane components can be washed away. Bound proteins can be eluted with soluble carbohydrate competitor or with urea or like

denaturing agent. Partial amino acid sequences from the purified protein can be used to make degenerate oligonucleotide probes to screen a cDNA library which can be sequenced to provide the full protein sequence. Once an intact cDNA is available and the protein sequence is known, it is then feasible to develop a strategy to overexpress the protein and initiate various structural studies to characterize the receptor.

In the preferred library of the invention, the glycosyl bond-forming step includes a condensation reaction between a glycosyl donor (GD) and a solid support-bound glycosyl acceptor (GA-SS) to provide a structural unit (GD-GA-SS) with a newly formed glycosyl bond. More preferably, the glycosyl bond-forming step includes a plurality of condensation reactions taking place substantially concurrently between a glycosyl donor and a plurality of distinct solid support-bound glycosyl acceptors to provide a plurality of distinct structural units with newly formed glycosyl bonds.

Alternatively, the glycosyl bond-forming step includes a plurality of condensation reactions taking place substantially concurrently between a plurality of distinct glycosyl donors and a solid support-bound glycosyl acceptor to provide a plurality of distinct structural units with newly formed glycosyl bonds; or, the glycosyl bond-forming step includes a plurality of condensation reactions taking place substantially concurrently between a plurality of distinct glycosyl donors and a plurality of distinct solid support-bound glycosyl acceptors to provide a plurality of structural units with newly formed glycosyl bonds. As described elsewhere herein, the plurality of condensation reactions can take place in the same reaction vessel or in separate reactions vessels.

Of very practical significance, the present

invention provides an assay (and method) for a carbohydrate-based ligand-receptor interaction comprising the steps of: (a) providing a library comprising a collection of distinct carbohydrate-based ligands, a plurality of each ligand being bound to and presented on the surface of a resolvable portion of a solid support; (b) contacting the library with one or more probes bearing a plurality of carbohydrate binding sites; and (c) selecting at least one particular ligand-probe interaction. The contacting step may be carried out in a vessel containing a plurality of members of the library.

In the assay of the invention, the selection step includes selecting those resolvable portions of the solid support to which a probe has bound. The assay is made possible by the library of the invention, which permits multivalent interactions of the plurality of ligands with the one or more probes. As mentioned earlier, a solid support may comprise an insoluble material, such as insoluble polymer. Preferred solid supports comprise a polystyrene resin or a polystyrene resin that is modified by covalently bound polyether chains. The solid support may also comprise a polyamide resin or one that is modified by covalently bound polyether chains. In a more preferred assay, the solid support comprises a polyethylene resin, a poly(ethylene glycol) resin, or a dendrimer polymer.

The assay probe may, of course, comprise one or more receptors, including receptors that are labeled with a detectable moiety, such as a radioisotope or a fluorescent or chemiluminescent substance. As one of ordinary skill in the art knows, the detectable moiety may comprise an enzyme that generates a detectable product.

Further, the detectable moiety may comprise a substance having a selective affinity for a detecting agent. For example, the substance may be biotin and

the detecting agent may be avidin or streptavidin.

In the assay of the present invention, the selection step may be carried out using an anti-probe or anti-ligand-probe antibody. Moreover, the antibody may be labeled with a detectable moiety, preferably a radioisotope, a fluorescent or chemiluminescent substance, an enzyme that generates a detectable product, a substance having a selective affinity for a detecting agent, or biotin.

In this manner, carbohydrate-based ligands with interesting properties and potential biological activity are selected by the assay of the invention. Such ligands can exhibit a variety of characteristics, including but not limited to those consistent with an enzyme inhibitor, a receptor agonist, a receptor antagonist, an antigen, an immunogen, an anti-tumor agent, an anticancer agent, an anti-emetic agent, an anti-inflammatory agent, a neurotransmitter, or a substance that exhibits endocrine-like properties.

In addition, the present invention contemplates a method of preparing a library comprising a collection of distinct carbohydrate-based ligands each bound to a resolvable portion of a solid support (SS) comprising (a) providing a plurality of distinct solid support-bound glycosyl acceptors (GA_1-SS, GA_2-SS, etc.), each distinct solid support-bound glycosyl acceptor being bound to a resolvable portion of a solid support, (b) contacting the plurality of distinct solid support-bound glycosyl acceptors with at least one distinct glycosyl donor (GD) such that condensation reactions take place, including glycosyl bond-forming steps, between the at least one distinct glycosyl donor and each of the distinct solid support-bound glycosyl acceptors to provide at least the distinct structural units (GD-GA_1-SS, GD-GA_2-SS, etc.). In particular, the plurality of distinct solid support-bound glycosyl acceptors may be

provided in separate reaction vessels each holding a distinct solid support-bound glycosyl acceptor.

In a particular method of the invention, at least one distinct glycosyl donor is contacted with each of the distinct solid support-bound glycosyl acceptors. Alternatively, the plurality of distinct solid support-bound glycosyl acceptors is *not* provided in separate reaction vessels each holding a distinct solid support-bound glycosyl acceptor. On the other hand, a particular method may involve contacting at least one distinct glycosyl donor with the plurality of distinct solid support-bound glycosyl acceptors substantially concurrently in the same reaction vessel. The method can further comprise contacting at least the distinct structural units (GD-GA_1-SS, GD-GA_2-SS, etc.) with one or more additional reagents, including one or more additional glycosyl donors.

7. Examples

The scope of the present invention is not limited in any way by the scope of the examples provided below, which are presented for illustrative purposes only.

7.1. Preparation of 1,6-Dideoxy-2,3,4-tri-*O*-pivaloyl-1-(phenylsulfinyl)-β-L-galactopyranose (5)

To a solution of L-fucose (1.03 g, 6.09 mmol) in 60 mL of pyridine at room temperature is added acetic anhydride (4.6 mL, 4.96 g, 48.7 mmol). The solution is stirred at room temperature for 20 h and concentrated *in vacuo*. The residue is then dissolved in 50 mL of CH_2Cl_2 and washed with 5% HCl (4 x 60 mL), dried over anhydrous Na_2SO_4 and concentrated to afford 1.76 g (85%) of L-fucose tetraacetate **1** as a white foam, a 5:2 (α:β) mixture of anomers: R_f 0.38 (40% petroleum ether/EtOAc); ^{1}H NMR ($CDCl_3$, 270 MHz, mixture of anomers) δ 6.33 (d, J = 2.0 Hz, 1H, H-1α),

5.67 (d, J = 8.2 Hz, 1H, H-1β), 5.26-5.38 (m, 5H), 5.06 (dd, J = 10.4, 3.4 Hz, 1H, H-3b), 4.27 (q, J = 6.6 Hz, 1H, H-5α), 3.95 (q, J = 6.3 Hz, 1H, H-5β), 2.23 (s, 3H), 2.19 (s, 3H), 2.18 (s, 3H), 2.17 (s, 3H), 2.15 (s, 3H), 2.04 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H), 1.22 (d, J = 6.6 Hz, 3H, H-6β), 1.16 (d, J = 6.6 Hz, 3H, H-6α).

To a solution of L-fucose tetraacetate **1** (1.76 g, 5.30 mmol) in 50 mL of CH_2Cl_2 is added thiophenol (1.4 mL, 1.46 g, 13.2 mmol) followed by $BF_3 \cdot OEt_2$ (3.3 mL, 3.76 g, 26.5 mmol). The reaction mixture is stirred at room temperature for 17 h and then quenched by the addition of 5 mL of H_2O. The reaction mixture is diluted with 200 mL of CH_2Cl_2, washed with H_2O (100 mL), saturated NaCl (100 mL), dried over Na_2SO_4, filtered and concentrated to afford 2.77 g of a clear oil, which is purified by flash chromatography (17% EtOAc/petroleum ether) to give 1.36 g (67%) of phenyl 6-deoxy-2,3,4-tri-*O*-acetyl-1-thio-L-galactopyranoside **2** as a 3.5:1 (β:α) mixture of anomers. The anomers could be separated by flash chromatography (17% EtOAc/petroleum ether): R_f (β-anomer) 0.19 (17% EtOAc/petroleum ether); R_f (α-anomer) 0.26 (17% EtOAc/petroleum ether); ^1H NMR ($CDCl_3$, 300 MHz) β-anomer, δ 7.41-7.55 (m, 2H), 7.22-7.37 (m, 3H), 5.20-5.30 (m, 2H), 5.05 (dd, J = 9.9, 3.3 Hz, 1H, H-3), 4.70 (d, J = 9.9 Hz, 1H, H-1), 3.84 (q, J = 6.4 Hz, 1H, H-5), 2.15 (s, 3H), 2.09 (s, 3H), 1.98 (s, 3H), 1.24 (d, J = 6.6 Hz, 3H, H-6); α-anomer, δ 7.48-7.53 (m, 2H), 7.16-7.45 (m, 3H), 5.93 (d, J = 5.1 Hz, 1H, H-1), 5.27-5.41 (m, 3H), 4.61 (q, J = 6.6 Hz, 1H, H-5), 2.17 (s, 3H), 2.11 (s, 3H), 2.02 (s, 3H), 1.13 (d, J = 6.6 Hz, 3H, H-6).

To a solution of phenyl 6-deoxy-2,3,4-tri-*O*-acetyl-1-thio-β-L-galactopyranoside **2** (0.892 g, 2.33 mmol) in 25 mL of methanol is added K_2CO_3 (0.644 g, 4.66 mmol). The reaction mixture is stirred at room temperature for 12 h. Amberlite resin (H^+ form) is

added to the reaction mixture and stirred for an additional 30 min. The neutralized mixture is then filtered through Celite, which is washed several times with methanol, and the filtrate is concentrated to afford 1.39 g of phenyl 6-deoxy-1-thio-β-L-galactopyranoside **3** as a yellow oil, which is used in the next step without further purification. The thioglycoside **3** can be purified using flash chromatography (10% $MeOH/CH_2Cl_2$): R_f 0.28 (10% $MeOH/CH_2Cl_2$); ^1H NMR (C_6D_6, 270 MHz) δ 7.54-7.58 (m, 2H), 7.28-7.37 (m, 3H), 4.50 (d, J = 8.2 Hz, 1H, H-1), 3.70 (q, J = 6.6 Hz, 1H, H-5), 3.56-3.75 (m, 3H), 1.38 (d, J = 6.6 Hz, 3H).

To a solution of crude phenyl 6-deoxy-1-thio-β-L-galactopyranoside **3** in 15 mL of pyridine is added pivaloyl chloride (4.0 mL, 3.90 g, 32.5 mmol) and DMAP (0.33 g, 2.71 mmol). The reaction mixture is heated at 100 °C for 12 h, cooled, diluted with 50 mL of CH_2Cl_2, washed with H_2O (100 mL) and saturated NaCl (100 mL), dried over Na_2SO_4, filtered and concentrated to afford 3.25 g of phenyl 6-deoxy-2,3,4-tri-*O*-pivaloyl-1-thio-β-L-galactopyranoside **4** as a brown oil. This oil is purified by flash chromatography (15% EtOAc/petroleum ether) to afford 0.53 g (48%) of the pure compound. An additional 0.81 g of a brown oil, which that appeared to be a mixture of incompletely acylated products, is also isolated. This material is resubjected to the reaction conditions for 48 h and provided an additional 0.21 g (19%, total yield 67% for 2 steps): R_f 0.48 (15% EtOAc/petroleum ether); ^1H NMR ($CDCl_3$, 270 MHz) δ 7.50-7.59 (m, 2H), 7.23-7.35 (m, 3H), 5.16-5.30 (m, 2H), 5.08 (dd, J = 9.9, 3.0 Hz, 1H, H-3), 4.66 (d, J = 9.6 Hz, 1H, H-1), 3.90 (q, J = 6.3 Hz, 1H, H-5), 1.21 (s, 9H), 1.18 (s, 9H), 1.21 (s, 9H), 1.21 (d, J = 6.6 Hz, 3H, H-6).

To a solution of phenyl 6-deoxy-2,3,4-tri-*O*-pivaloyl-1-thio-β-L-galactopyranoside **4** (2.47 g, 5.22

mmol) in 65 mL of CH_2Cl_2 at -78 °C is added mCPBA (1.53 g, 8.84 mmol). The reaction mixture is allowed to warm to -15 °C and then quenched with methyl sulfide (5.3 mL, 4.48 g, 17.4 mmol) and allowed to warm to room temperature. The reaction mixture is then diluted with 50 mL CH_2Cl_2, extracted with H_2O (100 mL), saturated $NaHCO_3$ (100 mL), saturated NaCl (100 mL), dried over Na_2SO_4, filtered and concentrated to afford a white solid. The sulfoxide is purified using flash chromatography (15% EtOAc/petroleum ether) to afford 1.81 g (66%) of 1,6-dideoxy-2,3,4-tri-*O*-pivaloyl-1-(phenylsulfinyl)-β-L-galactopyranose 5 as a mixture of diastereomers: R_f 0.05 and 0.13 (15% EtOAc/petroleum ether).

7.2. 1,6-Dideoxy-1-(phenylsulfinyl)-2,3,4-tri-*O*-pivaloyl-β-D-galactopyranose (β-D-5)

By the above method, but beginning with D-fucose, the title compound is prepared.

7.3. 3-Azido-4-*O*-benzoyl-1,3,6-trideoxy-2-*O*-pivaloyl-1-(phenylsulfinyl)-β-L-galactopyranose (20)

To a solution of L-fucose phenylthioglycoside (phenyl 6-deoxy-1-thio-L-galactopyranoside, 3, prepared above) (3.4 g, 13 mmol) in 250 mL of DMF is added p-toluenesulfonic acid hydrate (1.3 g, 6.6 mmol) and 2,2-dimethoxypropane (3.3 mL, 27 mmol). The reaction mixture is stirred at room temperature for 12 h and then quenched by the addition of 10 mL of saturated $NaHCO_3$. The reaction mixture is diluted with 400 mL of CH_2Cl_2, washed with saturated $NaHCO_3$ (2 x 200 mL), dried over Na_2SO_4, filtered, concentrated and purified by flash chromatography (8% EtOAc/petroleum ether) to give 3.3 g (85%) of phenyl 6-deoxy-3,4-*O*-isopropylidene-1-thio-L-galactopyranoside 6 as a mixture of anomers. The anomers could be separated

by flash chromatography: R_f (α-6) 0.58 (50% EtOAc/petroleum ether); R_f (β-anomer) 0.46 (50% EtOAc/petroleum ether); ^{1}H NMR of β-6 ($CDCl_3$, 300 MHz) δ 7.70-7.55 (m, 2H, ArH), 7.45-7.28 (m, 3H, ArH), 4.48 (d, J = 10 Hz, 1H, H-1), 4.15-4.10 (m, 2H, H-3 and H-4), 3.96 (q, J = 4.8 Hz, 1H, H-5), 3.62 (q, J = 4.0 Hz, 1H, H-2), 2.52 (s, br, 1H, OH), 1.53-1.50 (m, 6H, H-6 and CH_3), 1.42 (s, 3H, CH_3).

To a solution of phenyl 6-deoxy-3,4-*O*-isopropylidene-1-thio-L-galactopyranoside **6** mixed anomers (2.12 g, 7.16 mmol) in 140 mL of DMF is added NaH (568 mg, 14.2 mmol), and the reaction mixture stirred for 10 min. *p*-Methoxybenzyl chloride (1.9 mL, 14.2 mmol) is added, and the solution is stirred for 30 min and then quenched with 10 mL of saturated $NaHCO_3$. The reaction mixture is diluted with 300 mL of CH_2Cl_2, washed with saturated $NaHCO_3$ (150 mL), dried over Na_2SO_4, filtered and concentrated. The crude product is purified by flash chromatography (8% EtOAc/petroleum ether) to give 2.6 g (87%) of phenyl 6-deoxy-3,4-*O*-isopropylidene-2-(4-methoxybenzyl)-1-thio-L-galactopyranoside **7** as a mixture of anomers: R_f 0.50 (35% EtOAc/petroleum ether); ^{1}H NMR of β-7 ($CDCl_3$, 300 MHz) δ 7.60 (d, 2H, ArH), 7.43-7.28 (m, 5H, ArH), 6.94 (d, 2H, ArH), 4.82 (d, J = 10.6 Hz, 1H, CH_2), 4.67 (d, J = 10.6 Hz, 1H, CH_2), 4.65 (d, J = 9.9 Hz, 1H, H-1), 4.29 (t, J = 5.9 Hz, 1H, H-2), 4.13 (dd, J = 5.9, 2.2 Hz, 1H, H-3), 3.91-3.86 (m, 4H, H-4, OCH_3), 3.56 (dd, J = 6.2, 3.3 Hz, 1H, H-5), 1.50 (s, 3H, CH_3), 1.47 (d, J = 6.2 Hz,3H, H-6) 1.44 (s, 3H, CH_3).

To a solution of phenyl 6-deoxy-3,4-*O*-isopropylidene-2-(4-methoxybenzyl)-1-thio-L-galactopyranoside **7** mixed anomers (2.4 g, 5.8 mmol) in 60 mL of MeOH is added $TsOH{\cdot}H_2O$ (540 mg, 2.9 mmol). The reaction mixture is stirred at room temperature for 5 h and then neutralized with Amberlite resin (OH^- form). The solution is filtered, washed several

times with MeOH and then concentrated *in vacuo*. The product is purified by flash chromatography (60% EtOAc/petroleum ether) to give 2.06 g (95%) of isolated anomers of phenyl 6-deoxy-2-(4-methoxybenzyl)-1-thio-L-galactopyranoside **9**. ^1H NMR ($CDCl_3$, 300 MHz) of **β-9**: δ 7.65 (d, 2H, ArH), 7.40-7.37 (m, 5H, ArH), 6.96 (d, 2H, ArH), 4.95 (d, J = 10.6 Hz, 1H, CH_2), 4.69 (d, J = 10.6 Hz, 1H, CH_2), 4.67 (d, J = 9.5 Hz, 1H, H-1), 3.87 (s, 3H, OCH_3), 3.79 (d, J = 2.9 Hz, 1H, H-4), 3.72-3.58 (m, 3H, H-2, H-3, H-5), 2.46 (s, br, 2H, OH), 1.42 (d, J = 6.2 Hz, 3H, H-6); **α-9**, δ 7.56 (d, 2H, ArH), 7.43-7.31 (m, 5H, ArH), 6.95 (d, 2H, ArH), 5.78 (d, J = 5.7 Hz, 1H, H-1), 4.78 (d, J = 11.5 Hz, 1H, CH_2), 4.58 (d, J = 11.5 Hz, 1H, CH_2), 4.50 (q, J = 6.4 Hz, 1H, H-5), 4.12 (dd, J = 5.7, 9.6 Hz, 1H, H-3), 3.97-3.82 (m, 5H, H-2, H-4, OCH_3), 3.18 (s, br, 1H, OH), 2.88 (s, br, 1H, OH), 1.40 (d, J = 6.4 Hz, 3H, H-6).

Trifluoromethanesulfonic anhydride (2.5 mL, 15 mmol) is added dropwise to a cooled (0 °C) solution of phenyl 6-deoxy-2-(4-methoxybenzyl)-1-thio-β-L-galactopyranoside (**β-9**) (1.4 g, 3.8 mmol) and pyridine (3.0 mL, 38 mmol) in 50 mL of CH_2Cl_2. The solution is stirred at 0 °C for 45 min and is allowed to warm to room temperature over 3 h. The reaction mixture is then cooled to 0 °C before quenching with TEA (1 mL, 7.2 mmol). The reaction mixture is diluted with 25 mL of CH_2Cl_2, extracted with saturated $NaHCO_3$ (2 x 50 mL), dried over Na_2SO_4, filtered, concentrated and purified by flash chromatography (8% EtOAc/petroleum ether) to give phenyl 6-deoxy-3,4-di-*O*-trifluoromethanesulfonyl-2-(4-methoxybenzyl)-1-thio-β-L-galactopyranoside (**β-12**): R_f 0.44 (20% EtOAc/petroleum ether); ^1H NMR ($CDCl_3$, 270 MHz) δ 7.60-7.57 (m, 2H, ArH), 7.38-7.33 (m, 5H, ArH), 6.90 (d, 2H, ArH), 5.16 (d, J = 2.3 Hz, 1H, H-4), 4.92 (dd, J = 2.3, 9.6 Hz, 1H, H-3), 4.80 (d, J = 9.2 Hz, 1H, CH_2), 4.65 (d, J = 9.6 Hz, 1H, H-1), 4.63 (d, J =

9.2 Hz, 1H, CH_2), 3.83-3.76 (m, 5H, H-2, H-5, OCH_3), 1.45 (d, J = 6.6 Hz, 3H, H-6).

To a solution of the above bis(triflate) β-**12** in 40 mL of toluene is added potassium benzoate (1.8 g, 11 mmol) and 18-crown-6 (3.0 g, 11 mmol). The reaction mixture is stirred at room temperature for 5 h and then diluted with 40 mL of CH_2Cl_2. The resulting solution is washed with saturated $NaHCO_3$ (2 x 50 mL), dried over Na_2SO_4, filtered and concentrated. The crude phenyl 6-deoxy-3,4-di-*O*-benzoyl-2-*O*-(4-methoxybenzyl)-1-thio-β-L-allopyranoside (β-**13**) is used without further purification in the next step. However, the material can be purified by flash chromatography (10% EtOAC/petroleum ether): R_f 0.59 (20% EtOAc/petroleum ether); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 8.02 (d, 2H, ArH), 7.85 (d, 2H, ArH), 7.61-7.23 (m, 9H, ArH) 6.83 (d, 2H, ArH), 6.16 (t, J = 3.0 Hz, 1H, H-3), 5.17 (d, J = 9.6 Hz, 1H, H-1), 4.95 (dd, J = 2.6, 9.9 Hz, 1H, H-4), 4.66 (d, J = 10.9 Hz, 1H, CH_2), 4.37 (d, J = 10.9 Hz, 1H, CH_2), 4.24 (dq, J = 6.3, 9.9 Hz, 1H, H-5), 3.78 (s, 3H, OCH_3), 3.60 (dd, J = 3.0, 9.6 Hz, 1H, H-2), 1.33 (d, J = 6.3 Hz, 3H, H-6).

To a solution of crude phenyl 6-deoxy-3,4-di-*O*-benzoyl-2-*O*-(4-methoxybenzyl)-1-thio-β-L-allopyranoside β-**13** in 20 mL of MeOH is added NaOMe (200 mg, 3.8 mmol). The reaction mixture is stirred at room temperature for 2 h and then neutralized with Amberlite resin. The solution is filtered, washed several times with MeOH and concentrated *in vacuo*. The crude product is purified by flash chromatography (60% EtOAc/petroleum ether) to afford 1.01 g (72%, 3 steps) of phenyl 6-deoxy-2-*O*-(4-methoxybenzyl)-1-thio-β-L-allopyranoside (β-**14**): R_f 0.35 (50% EtOAc/petroleum ether); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 7.56-7.52 (m, 2H, ArH), 7.31-7.27 (m, 5H, ArH), 6.89-6.88 (m, 2H, ArH), 4.93 (d, J = 9.9 Hz, 1H, H-1), 4.71 (d, J = 11.2 Hz, 1H, CH_2), 4.55 (d, J = 11.2 Hz,

1H, CH_2), 4.13 (t, J = 2.6 Hz, 1H, H-3), 3.83 (s, 3H, OCH_3), 3.73-3.63 (m, 1H, H-5), 3.38 (dd, J = 3.3, 9.9 Hz, 1H, H-2), 3.21 (dt, J = 3.3, 9.6 Hz, 1H, H-4), 2.58 (s, 1H, OH) 2.34-2.30 (d, 1H, OH), 1.33 (d, J = 6.3 Hz, 3H, H-6).

Trifluoromethanesulfonic anhydride (1.8 mL, 10.8 mmol) is added at 0 °C to a solution of phenyl 6-deoxy-2-*O*-(4-methoxybenzyl)-1-thio-β-L-allopyranoside (β-**14**) (1.01 g, 2.69 mmol) and pyridine (2.2 mL, 26.9 mmol) in 30 mL of CH_2Cl_2. The solution is stirred at 0 °C for 1 h and allowed to warm at room temperature over 3 h. The reaction mixture is cooled to 0 °C before quenching with TEA (1.0 mL, 7.2 mmol). The reaction mixture is diluted with 30 mL of CH_2Cl_2, washed with saturated $NaHCO_3$ (30 mL), 1N HCl (30 mL), dried over Na_2SO_4, filtered and concentrated. The crude product is purified by flash chromatography (8% EtOAc/petroleum ether) to give 1.36 g (79%) of phenyl 6-deoxy-3,4-di-*O*-(trifluoromethanesulfonyl)-2-*O*-(4-methoxybenzyl)-1-thio-β-L-allopyranoside (β-**15**): R_f 0.49 (20% EtOAc/petroleum ether); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 7.48 (d, 2H, ArH), 7.33-7.21 (m, 5H, ArH), 6.91 (d, 2H, ArH), 5.34 (t, J = 2.0 Hz, 1H, H-3), 4.93 (d, J = 9.2 Hz, 1H, H-1), 4.77 (d, J = 11.2 Hz, 1H, CH_2), 4.65 (d, J = 11.2 Hz, 1H, CH_2), 4.55 (dd, J = 2.0, 8.8 Hz, 1H, H-4), 4.19-3.96 (m, 1H, H-5), 3.90 (s, 3H, OCH_3), 3.47 (dd, J = 2.0, 9.2 Hz, 1H, H-2), 1.36 (d, J = 5.8 Hz, 3H, H-6).

The above bis(triflate) β-**15** (297 mg, 2.13 mmol) is dissolved in 10 mL of DMF and cooled at -15 °C. To the solution is added NaN_3 (137 mg, 2.11 mmol) and the reaction mixture is stirred at -15 °C for 45 minutes. Potassium benzoate (511 mg, 3.20 mmol) and 18-crown-6 (844 mg, 3.20 mmol) are added to the reaction mixture. The reaction mixture is stirred for 8 h and is then diluted with 20 mL of CH_2Cl_2. The resulting solution is washed with $NaHCO_3$ (3 x 20 mL), dried over Na_2SO_4, filtered and concentrated. The

resulting phenyl 3-azido-4-*O*-benzoyl-3,6-di-deoxy-2-*O*-(4-methoxybenzyl)-1-thio-β-L-galactopyranoside (β-**16**) is purified by flash chromatography (20% EtOAc/petroleum ether) and taken on immediately to the next step: R_f 0.35 (20% EtOAc/petroleum ether); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 8.01 (d, 2H, ArH), 7.71-7.26 (m, 10H, ArH), 6.90 (d, 2H, ArH), 5.47 (d, *J* = 2.0 Hz, 1H, H-4), 4.91 (d, *J* = 9.6 Hz, 1H, CH_2), 4.69 (d, *J* = 9.2 Hz, 1H, H-1), 4.64 (d, *J* = 9.6 Hz, 1H, CH_2), 3.85-3.64 (m, 6H, H-2, H-3, H-5, OCH_3), 1.27 (d, *J* = 6.3 Hz, 3H, H-6).

To a solution of phenyl 3-azido-4-*O*-benzoyl-3,6-di-deoxy-2-*O*-(4-methoxybenzyl)-1-thio-β-L-galactopyranoside (β-**16**) in 50 mL of CH_2Cl_2 is added 5 mL of TFA (10% TFA/CH_2Cl_2). The reaction mixture is immediately diluted with 150 mL of CH_2Cl_2 and neutralized with $NaHCO_3$ (20 mL). The resulting suspension is washed with $NaHCO_3$ (100 mL), dried over Na_2SO_4, filtered and concentrated. The crude product is purified by flash chromatography (20% EtOAc/petroleum ether) to give 541 mg (66%, 2 steps) of phenyl 3-azido-4-*O*-benzoyl-3,6-di-deoxy-1-thio-β-L-galactopyranoside (β-**18**): R_f 0.21 (30% EtOAc/petroleum ether); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 7.88-7.85 (m, 2H, ArH), 7.68-7.56 (m, 3H, ArH), 7.47-7.35 (m, 5H, ArH), 5.44 (d, *J* = 2.3 Hz, 1H, H-4), 4.57 (d, *J* = 8.9 Hz, 1H, H-1), 3.92 (q, *J* = 6.3 Hz, 1H, H-5), 3.83 (dd, *J* = 2.3, 9.9 Hz, 1H, H-3), 3.74 (dt, *J* = 2.0, 9.9 Hz, 1H, H-2), 2.62 (d, *J* = 2.0, 1H, OH), 1.26 (d, *J* = 6.3 Hz, 3H, H-6).

To a solution of phenyl 3-a·.do-4-*O*-benzoyl-3,6-di-deoxy-1-thio-β-L-galactopyranoside (β-**18**) (118 mg, 0.307 mmol) in 5 mL of CH_2Cl_2 is added TEA (256 μL, 1.87 mmol), pivaloyl chloride (114 μL, 0.921 mmol) and DMAP (19 mg, 0.154 mmol). The reaction mixture is stirred at room temperature for 12 h, diluted with 15 mL of CH_2Cl_2, washed with 1M HCl (2 x 10 mL), dried over Na_2SO_4, filtered and concentrated. The crude

product is purified by flash chromatography (20% EtOAc/petroleum ether) to give 114 mg (80%) of phenyl 3-azido-4-*O*-benzoyl-3,6-di-deoxy-2-*O*-pivaloyl-1-thio-β-L-galactopyranoside (**β-19**): R_f 0.54 (20% EtOAc/petroleum ether); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 7.93 (d, 2H, ArH), 7.59-7.56 (m, 3H, ArH), 7.46-7.26 (m, 5H, ArH), 5.53 (d, J = 2.6 Hz, 1H, H-4), 5.25 (t, J = 9.9 Hz, 1H, H-2) 4.73 (d, J = 9.9 Hz, 1H, H-1), 3.92 (q, J = 6.3 Hz, 1H, H-5), 3.79 (dd, J = 2.6, 9.9 Hz, 1H, H-3), 1.42-1.21 (m, 12H, H-6, CH_3).

To a solution of phenyl 3-azido-4-*O*-benzoyl-3,6-di-deoxy-2-*O*-pivaloyl-1-thio-β-L-galactopyranoside (**β-19**) (270 mg, 0.575 mmol) in 10 mL of CH_2Cl_2 at -78 °C is added a solution of mCPBA (159 mg of 64% material, 0.920 mmol) in 2 mL of CH_2Cl_2. The reaction mixture is allowed to warm to -20 °C and then quenched with TEA (500 µL, 3.59 mmol). The reaction mixture is diluted with 10 mL of CH_2Cl_2, washed with saturated $NaHSO_3$ (10 mL), saturated $NaHCO_3$ (10 mL), dried over Na_2SO_4, filtered and concentrated. The crude product is purified by flash chromatography (60% EtOAc/petroleum ether) to give 231 mg (83%) of 3-azido-4-*O*-benzoyl-1,3,6-tri-deoxy-2-*O*-pivaloyl-1-(phenylsulfinyl)-β-L-galactopyranose (**β-20**) as a mixture of diastereomers: R_f 0.26 (50% EtOAc/petroleum ether).

7.4. 3-Azido-4-*O*-benzoyl-1,3,6-trideoxy-2-*O*-pivaloyl-1-(phenylsulfinyl)-β-D-galactopyranose (β-D-20)

By the method above, but beginning with D-fucose, the title compound is prepared.

7.5. 1,6-Dideoxy-2-(4-methoxybenzyl)-1-(phenylsulfinyl)-α-L-galactopyranose 3,4-carbonate (α-11)

To a solution of phenyl 6-deoxy-2-(4-methoxybenzyl)-1-thio-α-L-galacto-pyranoside (α-9, prepared above) (743 mg, 1.98 mmol) in 40 mL of THF is added 1,1'-carbonyldiimidazole (422 mg, 2.60 mmol). The reaction mixture is stirred at room temperature for 24 h and then quenched with 5 mL of 1M HCl. The resulting suspension is diluted with 40 mL of CH_2Cl_2, washed with of 1M HCl (15 mL), dried over Na_2SO_4, filtered and concentrated. The crude product is purified by flash chromatography (35% EtOAc/petroleum ether) to give 766 mg (97%) of phenyl 6-deoxy-2-(4-methoxybenzyl)-1-thio-α-L-galactopyranoside 3,4-carbonate (α-10): R_f 0.40 (40% EtOAc/petroleum ether); ^1H NMR ($CDCl_3$, 300 MHz) δ 7.50 (d, 2H, ArH), 7.38-7.32 (m, 5H, ArH), 6.96 (d, 2H, ArH), 5.74 (d, J = 5.6 Hz, 1H, H-1), 4.81 (d, J = 11.2 Hz, 1H, CH_2), 4.74 (dd, J = 5.1, 9.6 Hz, 1H, H-3), 4.67-4.60 (m, 2H, H-4 and CH_2), 4.53 (q, J = 6.1, 1H, H-5), 4.23-4.18 (m, 1H, H-2), 3.88 (s, 3H, CH_3), 1.35 (d, J = 6.1 Hz, 3H, H-6).

To a solution of phenyl 6-deoxy-2-(4-methoxybenzyl)-1-thio-α-L-galactopyranoside 3,4-carbonate (α-10) (280 mg, 0.691 mmol) in 10 mL of CH_2Cl_2 at -78 °C is added a solution of mCPBA (173 mg, 1.11 mmol) in 2 mL of CH_2Cl_2. The reaction mixture is allowed to warm to -20 °C and then quenched with TEA (500 μL, 3.59 mmol). The reaction mixture is diluted with 10 mL of CH_2Cl_2, washed with saturated $NaHSO_3$ (10 mL), saturated $NaHCO_3$ (10 mL), dried over Na_2SO_4, filtered and concentrated. The crude product is purified by flash chromatography (60% EtOAc/petroleum ether) to give 266 mg (92%) of 1,6-dideoxy-2-(4-methoxybenzyl)-1-(phenylsulfinyl)-α-L-galactopyranose 3,4-carbonate (α-11) as a mixture of diastereomers: R_f 0.26 (50% EtOAc/petroleum ether).

7.6. 1,6-Dideoxy-2-(4-methoxybenzyl)-1-(phenylsulfinyl)-α-D-galactopyranose 3,4-carbonate (α-D-11)

By the method above, but beginning with D-fucose, the title compound is prepared.

7.7. 1,6-Dideoxy-3,4-*O*-isopropylidene-2-(4-methoxybenzyl)-1-(phenylsulfinyl)-β-L-galactopyranose (8)

To a solution of phenyl 6-deoxy-3,4-*O*-isopropylidene-2-(4-methoxybenzyl)-1-thio-L-galactopyranoside (7, prepared above), (290 mg, 0.69 mmol) in 10 mL of CH_2Cl_2 at -78 °C is added a solution of mCPBA (170 mg, 1.1 mmol) in 2 mL of CH_2Cl_2. The reaction mixture is allowed to warm to -20 °C and then quenched with TEA (500 µL, 3.59 mmol). The reaction mixture is diluted with 10 mL of CH_2Cl_2, washed with saturated $NaHSO_3$ (10 mL), saturated $NaHCO_3$ (10 mL), dried over Na_2SO_4, filtered and concentrated. The crude product is purified by flash chromatography (EtOAc/petroleum ether) to give 1,6-dideoxy-3,4-*O*-isopropylidene-2-(4-methoxybenzyl)-1-(phenylsulfinyl)-L-galactopyranose (8) as a mixture of diastereomers.

7.8. 1,6-Dideoxy-3,4-*O*-isopropylidene-2-(4-methoxybenzyl)-1-(phenylsulfinyl)-D-galactopyranose (D-8)

By the method above, but beginning with D-fucose, the title compound is prepared.

7.9. 3,4-Di-*O*-benzoyl-1,6-dideoxy-2-*O*-(4-methoxybenzyl)-1-(phenylsulfinyl)-β-L-allopyranose (β-13 sulfoxide)

To a solution of phenyl 6-deoxy-3,4-di-*O*-benzoyl-2-*O*-(4-methoxybenzyl)-1-thio-β-L-allopyranoside (**β-13**, prepared above) (400 mg, 0.7 mmol) in 10 mL of CH_2Cl_2 at -78 °C is added a solution of mCPBA (170 mg, 1.1 mmol) in 2 mL of CH_2Cl_2. The reaction mixture is allowed to warm to -20 °C and

then quenched with TEA (500 µL, 3.59 mmol). The reaction mixture is diluted with 10 mL of CH_2Cl_2, washed with saturated $NaHSO_3$ (10 mL), saturated $NaHCO_3$ (10 mL), dried over Na_2SO_4, filtered and concentrated. The crude product is purified by flash chromatography (EtOAc/petroleum ether) to give 1,6-dideoxy-3,4-di-*O*-benzoyl-2-*O*-(4-methoxybenzyl)-1-(phenylsulfinyl)-β-L-allopyranose as a mixture of diastereomers.

7.10. 3,4-Di-*O*-benzoyl-1,6-dideoxy-2-*O*-(4-methoxybenzyl)-1-(phenylsulfinyl)-β-D-allopyranose (β-D-13 sulfoxide)

By the method above, but beginning with D-fucose, the title compound is prepared.

7.11. 3-Azido-4-*O*-benzoyl-1,3,6-trideoxy-2-*O*-(4-methoxybenzyl)-1-(phenylsulfinyl)-β-L-galactopyranose (β-17)

To a solution of phenyl 3-azido-4-*O*-benzoyl-3,6-di-deoxy-2-*O*-(4-methoxybenzyl)-1-thio-β-L-galactopyranoside (**β-16**, prepared above), (350 mg, 0.7 mmol) in 10 mL of CH_2Cl_2 at -78 °C is added a solution of mCPBA (170 mg, 1.1 mmol) in 2 mL of CH_2Cl_2. The reaction mixture is allowed to warm to -20 °C and then quenched with TEA (500 µL, 3.59 mmol). The reaction mixture is diluted with 10 mL of CH_2Cl_2, washed with saturated $NaHSO_3$ (10 mL), saturated $NaHCO_3$ (10 mL), dried over Na_2SO_4, filtered and concentrated. The crude product is purified by flash chromatography (EtOAc/petroleum ether) to give the title compound as a mixture of diastereomers.

7.12. 3-Azido-4-*O*-benzoyl-1,3,6-trideoxy-2-*O*-(4-methoxybenzyl)-1-(phenylsulfinyl)-β-D-galactopyranose (β-D-17)

By the method above, but beginning with D-fucose, the title compound is prepared.

7.13. 2-Azido-1,2,6-trideoxy-1-(phenylsulfinyl)-α-L-galactopyranose 3,4-carbonate

(α-25)

The mixed anomers of 1,3,4-tri-*O*-acetyl-2-azido-2,6-dideoxy-L-galactopyranoside (**21**) are prepared from L-fucose by the method of A. Anisuzzaman and D. Horton, *Carb. Res.*, **169**, 258-262 (1987). To a solution of **21** (3.80 g, 12.1 mmol) in 120 mL of CH_2Cl_2, thiophenol (3.1 mL, 3.3 g, 30.1 mmol) and $BF_3 \cdot Et_2O$ (7.4 mL, 8.5 g, 60.3 mmol) are added at room temperature. The reaction mixture is heated at 40 °C for 50 min, then quenched with H_2O (20 mL). The organic layer is washed with brine (100 mL), dried over Na_2SO_4, filtered and concentrated to afford a mixture of anomers of phenyl 2-azido-3,4-di-*O*-acetyl-2,6-dideoxy-1-thio-L-galactopyranoside (**22**) as a clear oil: R_f 0.39 (25% EtOAc/petroleum ether).

To a solution of **22** (4.40 g, 12.1 mmol) in 120 mL of methanol is added K_2CO_3 (5.97 g, 36.2 mmol). The reaction mixture is stirred at room temperature for 1.5 h and then neutralized with amberlite resin, filtered, concentrated and purified by flash chromatography (5% methanol/CH_2Cl_2) to yield 3.10 g (91%, 2 steps) of phenyl 2-azido-2,6-dideoxy-1-thio-L-galactopyranoside (**23**) as a 2.5:1 (α:β) mixture of anomers: R_f 0.10 (25% EtOAc/petroleum ether); ^1H NMR ($CDCl_3$, 270 MHz) mixture of anomers, δ 7.61-7.27 (m, 10H), 5.61 (d, J = 5.3 Hz, 1H, H-1α), 4.50 (q, J = 6.6 Hz, 1H, H-5α), 4.42 (d, J = 9.9 Hz, 1H, H-1β), 4.11 (dd, J = 10.1, 5.4 Hz, 2H, H-3α,β), 3.88-3.85 (m, 3H), 3.73 (br s, 1H), 3.63 (q, J = 6.3 Hz, 1H, H-5β), 3.59-3.40 (m, 1H), 2.81 (br s, 1H, OH), 2.47 (br s, 1H, OH), 2.23 (br s, 1H, OH), 1.72 (br s, 1H, OH), 1.37 (d, J = 6.3 Hz, 3H, H-6β), 1.30 (d, J = 6.6 Hz, 3H, H-6α).

To a solution of **23** (2.90 g, 10.3 mmol) in 100 mL of CH_2Cl_2 at 0 °C is added 1,1'-carbonyldiimidazole (3.34 g, 20.6 mmol). The reaction mixture is allowed to warm to room temperature for 15 min and quenched with 50 mL of H_2O. The aqueous layer is extracted

with CH_2Cl_2 (3 x 40 mL), and the organic layers are combined, dried over Na_2SO_4, filtered, concentrated and purified by flash chromatography (gradient elution with 15-25% EtOAc/petroleum ether) to yield 2.30 g (73%) of phenyl 2-azido-2,6-dideoxy-1-thio-α-L-galactopyranoside 3,4-carbonate **24** as a white foam: R_f 0.38 (25% EtOAc/petroleum ether). The β-anomer, R_f 0.22, is not isolated. ^1H NMR ($CDCl_3$, 270 MHz) (α-**24**): δ 7.52-7.32 (m, 5H), 5.64 (d, *J* = 5.6 Hz, 1H, H-1), 4.90 (dd, *J* = 7.3, 5.6 Hz, 1H, H-3), 4.66 (dd, *J* = 7.6, 2.0 Hz, 1H, H-4), 4.47 (dq, *J* = 6.6, 2.0 Hz, 1H, H-5), 4.30 (app t, *J* = 5.6 Hz, 1H, H-2), 1.36 (d, *J* = 6.6 Hz, 3H, H-6).

To a solution of phenyl 2-azido-2,6-dideoxy-1-thio-α-L-galactopyranoside 3,4-carbonate (α-**24**) (0.860 g, 2.80 mmol) in 50 mL of CH_2Cl_2 is added $NaHCO_3$ at room temperature. The reaction mixture is then cooled to -78 °C, and mCPBA (0.878 g, 2.80 mmol, 50-60%) is added. The reaction mixture is stirred at -78 °C for 30 minutes and then allowed to warm to -40 °C over 1 h. The reaction is quenched with dimethyl sulfide (1 mL) at -40 °C and then poured into a solution of saturated $NaHCO_3$ (50 mL) and extracted with CH_2Cl_2 (3 x 30 mL). The organic layers are combined, dried over Na_2SO_4, filtered, concentrated and purified by flash chromatography (40% EtOAc/petroleum ether) to afford 0.871 g (96%) of 2-azido-1,2,6-trideoxy-1-(phenylsulfinyl)-α-L-galactopyranose 3,4-carbonate (α-**25**) as a mixture of diastereomers: R_f (major diastereomer) 0.14 (40% EtOAc/petroleum ether).

7.14. 2-Azido-1,2,6-trideoxy-1-(phenylsulfinyl)-α-D-galactopyranose 3,4-carbonate (α-D-25)

By the method above, but beginning with D-fucose, the title compound is prepared.

7.15. 2-Azido-3,4-di-*O*-acetyl-1,2,6-trideoxy-1-(phenylsulfinyl)-L-galactopyranose (26)

To a solution of phenyl 2-azido-3,4-di-*O*-acetyl-2,6-dideoxy-1-thio-L-galactopyranoside **(22)** (1.02 g, 2.80 mmol) in 50 mL of CH_2Cl_2 is added $NaHCO_3$ at room temperature. The reaction mixture is cooled to -78 °C, and mCPBA (0.878 g, 2.80 mmol, 50-60%) is added. The reaction mixture is stirred at -78 °C for 30 minutes and then allowed to warm to -40 °C over 1 h. The reaction is quenched with dimethyl sulfide (1 mL) at -40 °C and then poured into a solution of saturated $NaHCO_3$ (50 mL) and extracted with CH_2Cl_2 (3 x 30 mL). The organic layers are combined, dried over Na_2SO_4, filtered, concentrated, and purified by flash chromatography (EtOAc/petroleum ether) to afford the title compound **26** as a mixture of diastereomers.

7.16. 2-Azido-3,4-di-*O*-acetyl-1,2,6-trideoxy-1-(phenylsulfinyl)-D-galactopyranose (D-26)

By the method above, but beginning with D-fucose, the title compound is prepared.

7.17. 2,6-Bis-*O*-(4-methoxybenzyl)-1-deoxy-1-(phenylsulfinyl)-β-D-galactopyranose 3,4-carbonate (33)

By the method of A. Sarkar, K. Matta, *Carbohydr. Res.*, **233**, 245-250 (1992), 1,2,3,4,5,6-penta-*O*-acetyl-β-D-galactopyranose is converted into phenyl 1-thio-β-D-galactopyranoside **(27)**.

To a solution of **27** (5.3 g, 20 mmol) in 100 mL of DMF is added 2,2-dimethoxypropane (6.0 mL, 49 mmol) and *p*-toluenesulfonic acid monohydrate (0.74 g, 3.9 mmol). The reaction is stirred at room temperature for 2 days and then diluted with water (150 mL) and extracted with CH_2Cl_2 (3 X 150 mL). The organic layers are combined, dried over Na_2SO_4 and concentrated *in vacuo*. The residue is purified by flash chromatography (100% EtOAc) to give 4.9 g (80%) of phenyl 3,4-*O*-isopropylidine-1-thio-β-D-

galactopyranoside **28**, R_f 0.50 (100% EtOAc). ^1H NMR ($CDCl_3$, 270 MHz) δ 7.51-7.55 (m, 2H), 7.29-7.33 (m, 3H), 4.47 (d, *J* = 9.9 Hz, 1H, H-1), 4.18 (dd, *J* = 5.27, 1.65 Hz, 1H), 4.11 (appt, *J* = 6.3 Hz, 1H), 3.95-4.03 (m, 1H), 3.78-3.90 (m, 2H), 3.57 (dd, *J* = 10.2, 6.9 Hz, 1H), 1.41 (s, 3H), 1.33 (s, 3H).

To a solution of **28** (4.9 g, 16 mmol) in 120 mL of DMF at 0 °C is added NaH (1.5 g, 63 mmol). The solution is allowed to warm to room temperature over 0.5 h, and then *p*-methoxybenzyl chloride (8.5 mL, 63 mmol) and tetrabutylammonium iodide (4.1 g, 11 mmol) are added. After 4 h the reaction is quenched by pouring slowly into 500 mL of ice cold saturated $NaHCO_3$ with stirring. The product is extracted with CH_2Cl_2 (3 X 200 mL). The organic layers are dried over Na_2SO_4 and concentrated *in vacuo* to provide phenyl 2,6-bis-*O*-(4-methoxybenzyl)-3,4-*O*-isopropylidine-1-thio-β-D-galactopyranoside **29**, which is purified by flash chromatography (60% EtOAc/petroleum ether): R_f 0.51; ^1H NMR ($CDCl_3$, 270 MHz) δ 7.52-7.56 (m, 2H), 7.17-7.36 (m, 7H), 6.83-6.88 (m, 4H), 4.75 (d, *J* = 10.9 Hz, 1H), 4.42-4.69 (m, 4H), 4.18-4.26 (m, 2H), 3.88-3.93 (m, 1H), 3.75-3.80 (m, 8H), 3.49-3.57 (m, 1H), 1.42 (s, 3H), 1.35 (s, 3H).

The ketal **29** is dissolved in 100 mL of MeOH and *p*-toluenesulfonic acid monohydrate (0.60 g, 3.1 mmol) is added. The reaction is stirred at room temperature for 10 h and then saturated $NaHCO_3$ (50 mL) is added, followed by water (100 mL). The product is extracted with CH_2Cl_2 (4 X 100 mL), and the organic layers are combined, washed with saturated NaCl, (1 X 300 mL), dried over Na_2SO_4 and concentrated *in vacuo*. The residue is purified by flash chromatography (50% EtOAc/petroleum ether) to give 5.4 g (68% over 2 steps) of phenyl 2,6-bis-*O*-(4-methoxybenzyl)-1-thio-β-D-galactopyranoside **31**: R_f 0.26 (50% EtOAc/petroleum ether), ^1H NMR ($CDCl_3$, 270 MHz) δ

7.56-7.60 (m, 2H), 7.22-7.33 (m, 7H), 6.85-6.91 (m, 4H), 4.87 (d, J = 10.6 Hz, 1H, H-1), 4.58-4.65 (m, 2H), 4.50 (s, 2H), 4.02-4.03 (m, 1H), 3.81 (s, 3H), 3.80 (s, 3H), 3.75-3.77 (m, 2H), 3.59-3.62 (m, 3H).

To a solution **31** (5.4 g, 10 mmol) in 400 mL of CH_2Cl_2 at 0 °C is added carbonyldiimidazole (2.5 g, 15 mmol). The solution is stirred at 0 °C for 20 min and then for 12 h at room temperature. The solution is then quenched by the addition of brine (100 mL). The organic layer is dried over Na_2SO_4, and concentrated *in vacuo*. The residue is purified by flash chromatography (40% EtOAc/petroleum ether) to give 5.3 g (93%) of phenyl 2,6-bis-*O*-(4-methoxybenzyl)-1-thio-β-D-galactopyranoside 3,4-carbonate **32** as a syrup: R_f 0.69 (50% EtOAc/petroleum ether); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 7.48-7.51 (m, 2H), 7.22-7.31 (m, 7H), 6.87-6.91 (m, 4H), 4.77-4.83 (m, 3H), 4.63 (s, 2H), 4.48 (s, 2H), 3.96 (appt, J = 6.26 Hz, 1H), 3.82 (s, 6H), 3.67-3.75 (m, 3H).

To a solution of **32** (0.80 g 1.5 mmol) in 50 mL of CH_2Cl_2 at -78 °C is added 64% mCPBA (0.530 g, 1.96 mmol). The reaction is allowed to warm to -30 °C and quenched with dimethyl sulfide (0.3 mL, 4 mmol). Saturated $NaHCO_3$ (100 mL) is added and the product extracted with CH_2Cl_2 (2 X 150 mL). The organic layers are combined, dried over Na_2SO_4 and concentrated *in vacuo*. The residue is purified by flash chromatography (50% EtOAc/petroleum ether) to give 0.81 g (99%) of 2,6-bis-*O*-(4-methoxybenzyl)-1-deoxy-1-(phenylsulfinyl)-β-D-galactopyranose 3,4-carbonate **33** (a mixture of diastereomers) as a white foam: R_f 0.19 (50% EtOAc/petroleum ether).

7.18. 2,6-Bis-*O*-(4-methoxybenzyl)-3,4-*O*-isopropylidine-1-deoxy-1-(phenylsulfinyl)-β-D-galactopyranose (30)

To a solution of **29** (1.5 mmol) in 50 mL of CH_2Cl_2 at -78 °C is added 64% mCPBA (0.530 g, 1.96 mmol). The reaction is allowed to warm to -30 °C and

quenched with dimethyl sulfide (0.3 mL, 4 mmol). Saturated $NaHCO_3$ (100 mL) is added, and the product is extracted with CH_2Cl_2 (2 X 150 mL). The organic layers are combined, dried over Na_2SO_4 and concentrated *in vacuo*. The residue is purified by flash chromatography (EtOAc/petroleum ether) to give the title compound as a mixture of diastereomers.

7.19. 2,3,4,6-tetra-*O*-pivaloyl-1-deoxy-1-(phenylsulfinyl)-α-D-mannopyranose (37)

To a solution of D-mannose **34** (1.1 g, 6.1 mmol) in 30 mL of pyridine at room temperature is added pivaloyl chloride (4.5 mL, 36.6 mmol). The solution is heated to 100°C for 48 hr and then allowed to cool. The solvent is removed *in vacuo* and the residue is dissolved in 50 mL of CH_2Cl_2 and washed with 5% HCl (4 x 60 mL), dried over Na_2SO_4, filtered, and concentrated to afford a clear oil which is purified by flash chromatography (10% EtOAc/hexane) to give 3.6 g (97%) of penta(pivaloyl) D-mannose **35**, as a white solid, a 6:1 (α:β) mixture of anomers: R_f (β-anomer) 0.18 (10% EtOAc/hexane); R_f (α-anomer) 0.1 (10% EtOAc/hexane); ^1H NMR ($CDCl_3$, 300 MHz) δ 6.01 (d, *J* = 2.0Hz, 1H, H-1β), 5.83 (d, *J* = 1.0Hz, 1H, H-1α), 5.54-5.35 (m, 4H), 5.31-5.27 (m, 1H), 5.16 (dd, *J* = 10.2, 3.0Hz, 1H, H-3α), 4.20-4.13 (m, 4H), 4.05-3.97 (m, 1H, H-5β), 3.87-3.81 (m, 1H, H-5α), 1.30 (s, 9H), 1.29 (s, 9H), 1.28 (s, 9H), 1.23 (s, 9H), 1.22 (s, 9H), 1.17 (s, 18H), 1.16 (s, 9H), 1.13 (s, 9H), 1.12 (s, 9H).

To a solution of **35** (3.6 g, 6.0 mmol) in 40 mL of CH_2Cl_2 is added thiophenol (0.71 mL, 6.9 mmol) followed by boron trifluoride diethyl ether complex (2.95 mL, 24 mmol). The reaction mixture is stirred at room temperature for 10 hr and then quenched by the addition of 20 ml of saturated $NaHCO_3$ solution. The reaction mixture is then extracted with CH_2Cl_2 (3 x 30mL). The combined organic layers are dried over

Na_2SO_4, concentrated, and purified by flash chromotography (8% EtOAc/hexane) to give 3.1 g (85%) of phenyl 2,3,4,6-tetra-*O*-pivaloyl-1-thio-α-D-mannopyranoside **36** as a white solid: R_f 0.3 (8% EtOAc/hexane); ^1H NMR ($CDCl_3$, 300 MHz) δ 7.54-7.40 (m, 2H), 7.30-7.22 (m,4H), 5.50 (m, 2H, H-4, H-2), 5.39 (d, *J* = 1.3Hz, 1H, H-1), 5.29 (dd, *J* = 10.2, 3.3Hz, 1H, H-3), 4.62 (m, 1H, H-5), 4.22 (dd, *J* = 12.5, 4.3Hz, 1H, H-6), 4.10 (dd, *J* = 12.5, 1.3Hz, 1H, H-6), 1.26 (s, 9H), 1.21 (s, 9H), 1.18 (s, 9H), 1.14 (s, 9H).

To a solution of **36** (1.38 g, 2.27 mmol) in 65 mL of CH_2Cl_2 at -78 °C is added *m*-CPBA (612 mg, 64%, 2.27 mmol). The reaction mixture is allowed to warm to -15 °C and then quenched with dimethyl sulfide (1 mL, 13.6 mmol) and allowed to warm to room temperature. The reaction mixture is diluted with 50 mL of CH_2Cl_2, extracted with H_2O (100 mL), saturated $NaHCO_3$ (100 mL), saturated NaCl (100 mL), dried over Na_2SO_4, filtered, and concentrated to afford a white solid. The sulfoxide is purified using flash chromatography (30% EtOAc/hexane) to afford 1.07 g (76%) of the title compound **37** as a mixture of diastereomers: R_f 0.35 and 0.38 (30% EtOAc/hexane).

7.20. 1-deoxy-1-(phenylsulfinyl)-2,3,4,6-tetra-*O*-pivaloyl-β-D-galactopyranose (41)

To a solution of galactose pentaacetate (5.00 g, 12.8 mmol) in 125 mL of CH_2Cl_2 is added thiophenol (1.49 mL, 14.1 mmol) and boron trifluoride diethyl ether complex (4.73 mL, 38.4 mmol). The reaction mixture is stirred at room temperature for 5 h and then poured into 200 mL of ice water. The organic layer is washed with saturated $NaHCO_3$ (200 ml), dried over Na_2SO_4, filtered, concentrated and purified by flash chromatography (35% EtOAc/hexane) to give 5.4 g (96%) of 1-deoxy-1-(phenylthio)-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranose **39**: R_f

0.22 (35% EtOAc/hexane); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 7.35-7.65 (m, 5 H, ArH), 5.50 (br d, J = 3.3 Hz, 1 H, H-4), 5.32 (t, J = 9.8 Hz, 1 H, H-2), 5.13 (dd, J = 9.8, 3.3 Hz, 1 H, H-3), 4.80 (d, J = 9.8 Hz, 1 H, H-1), 4.13-4.32 (m, 2 H, H-6, H-6'), 4.02 (br t, J = 6.5 Hz, 1 H, H-5), 2.02-2.23 (4s, 12 H, OAc).

To a solution of thioglycoside **39** (1.10 g, 2.5 mmol) in 50 mL of MeOH is added K_2CO_3 in small portions, until the reaction mixture tested basic to pH paper (pH 11). The reaction mixture is stirred at room temperature for 15 min and then neutralized with Amberlite resin (acid form). The resin is removed by filtration and washed with MeOH (2 x 50 mL). The filtrates are concentrated and azeotroped from toluene to remove the residue MeOH to afford 1-deoxy-1-(phenylthio)-β-D-galactopyranose which is taken on to the next step without further purification.

To a solution of 1-deoxy-1-(phenylthio)-β-D-galactopyranose (0.38 g, 1.4 mmol) in 25 mL of pyridine, pivaloyl chloride (1.75 mL, 14.0 mmol) and DMAP (0.200 g, 1.40 mmol) are added. The reaction mixture is stirred at 90-100 °C for 8 h. Pyridine is removed under reduced pressure, and the residue is dissolved in 100 mL of CH_2Cl_2 and washed with dilute HCl (100 mL), H_2O (100 mL), dried over Na_2SO_4, filtered, concentrated and purified by flash chromatography (10% EtOAc/hexane) to give 0.65 g (76%) of 1-deoxy-1-(phenylthio)-2,3,4,6-tetra-*O*-pivaloyl-β-D-galactopyranose **40**: R_f 0.59 (15% EtOAc/hexane); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 7.97 (d, J = 8.9 Hz, 2 H, ArH), 7.70 (t, J = 7.3 Hz, 1 H, ArH), 7.60 (t, J = 7.3 Hz, 2 H, ArH), 5.39 (t, J = 9.9 Hz, 1 H, H-2), 5.30 (d, J = 3.0 Hz, 1 H, H-4), 5.10 (dd, J = 9.9, 3.0 Hz, 1 H, H-3), 4.57 (d, J = 9.9 Hz, 1 H, H-1), 4.05-4.20 (m, 2 H, H-6), 3.79 (dd, J = 10.6, 5.9 Hz, 1 H, H-5), 1.25 (s, 9 H), 1.18 (s, 9 H), 1.08 (s, 9H), 0.92 (s, 9 H). ^{13}C NMR ($CDCl_3$, 270 MHz) δ 177.7, 177.0, 176.7, 176.3, 134.4, 134.2, 131.0,

128.7, 89.4, 74.9, 71.7, 65.9, 63.9, 60.5, 38.4, 38.7, 38.6, 27.1.

To a solution of thioglycoside **40** (0.62 g, 1.0 mmol) in 20 mL of CH_2Cl_2 at -78 °C is added 67% *m*-CPBA (0.26 g, 1.0 mmol). The reaction mixture is stirred at -78 °C for 2 h and then allowed to slowly warm up to room temperature. Saturated $NaHCO_3$ is added until the solution is basic. The reaction mixture is diluted with 100 mL of CH_2Cl_2 and washed with H_2O (100 mL), saturated NaCl (100 mL), dried over Na_2SO_4, filtered, concentrated and purified by flash chromatography (15% EtOAc/hexane) to give 0.59 g (92%) of the title compound **41** as a mixture of diastereomers: R_f 0.38, 0.19 (20% EtOAc/hexane).

7.21. 2,3,4,6-tetra-*O*-pivaloyl-1-deoxy-1-(phenylsulfinyl)-β-D-glucopyranose (45)

To a solution of D-glucose **42** (500 mg, 2.77 mmol) in 25 mL of pyridine is added pivaloyl chloride (3.42 mL, 3.34 g, 27.75 mmol) followed by DMAP (34 mg, 0.27 mmol). The reaction mixture is heated at 100 °C for 12 h, cooled to room temperature and quenched with 2 mL methanol. The reaction mixture is concentrated and the resulting residue is taken up in 25 mL CH_2Cl_2, washed with 1N HCl (3 x 25 mL), saturated NaCl (25 mL), dried over Na_2SO_4, filtered, concentrated and purified by flash chromatography (10% EtOAc/hexane) to afford 1.49 g (90%) of 1,2,3,4,6-penta-*O*-pivaloyl-β-D-glucopyranose **43**: R_f 0.18 (5% EtOAc/hexane); ^1H NMR ($CDCl_3$, 270 MHz) δ 5.69 (d, J = 8.2 Hz, 1H, H-1), 5.37 (app t, J = 9.2 Hz, 1H, H-3), 5.21 (app t, J = 8.7 Hz, 1H, H-4), 5.16 (app t, J = 9.5 Hz, 1H, H-2), 4.06-4.17 (m, 2H, H-6), 3.86 (ddd, J = 9.8, 4.8, 2.3 Hz, 1H, H-5), 1.21 (s, 9H), 1.17 (s, 9H), 1.15 (s, 9H), 1.12 (s, 18H).

To a solution of **43** (1.49 g, 2.49 mmol) in 50 mL CH_2Cl_2 is added thiophenol (0.64 mL, 686 mg, 6.22 mmol) followed by boron trifluoride diethyl

ether complex (1.53 mL, 1.76 g, 12.45 mmol). The reaction mixture is stirred at room temperature for 4 h and quenched by the slow addition of 10 mL saturated $NaHCO_3$ solution. The reaction mixture is diluted with 100 mL of CH_2Cl_2, washed with H_2O (100 mL), saturated NaCl (100 mL), dried over Na_2SO_4, filtered, concentrated and purified by flash chromatography (5% EtOAc/hexane) to afford 1.24 g (82%) of 1-deoxy-1-(phenylthio)-2,3,4,6-tetra-*O*-pivaloyl-β-D-glucopyranose **44**: R_f 0.25 (5% EtOAc/hexane); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 7.47-7.50 (m, 2H), 7.22-7.30 (m, 3H), 5.34 (app t, *J* = 9.4 Hz, 1H, H-3), 5.08 (app t, *J* = 9.7 Hz, 1H, H-4), 5.03 (app t, *J* = 9.7 Hz, 1H, H-2), 4.73 (d, *J* = 9.8 Hz, 1H, H-1), 4.25 (dd, *J* = 12.2, 1.6 Hz, 1H, H-6), 4.04 (dd, *J* = 12.2, 5.9 Hz, 1H, H-6), 3.76 (ddd, *J* = 10.2, 5.9, 1.6 Hz, 1H, H-5), 1.21 (s, 9H), 1.20 (s, 9H), 1.14 (s, 9H), 1.10 (s, 9H).

To a solution of **44** (1.24 g, 2.04 mmol) in 25 mL of CH_2Cl_2 at -78 °C is added a solution of 64% *m*-CPBA (550 mg, 2.04 mmol) in 10 mL of CH_2Cl_2. The reaction mixture is allowed to warm to -15 °C, quenched with methyl sulfide (2.07 mL, 1.75 g, 6.8 mmol) and warmed to room temperature. The reaction mixture is then diluted with 25 mL CH_2Cl_2, washed with saturated $NaHCO_3$ (50 mL), H_2O (50 mL), saturated NaCl (50 mL), dried over Na_2SO_4, filtered, concentrated and purified by flash chromatography (30% EtOAc/hexane) to afford 1.14 g (89%) of the title compound **45** as a mixture of diastereomers: R_f 0.14 and 0.28 (10% EtOAc/hexane).

7.22. 2,3,4,6-tetra-*O*-pivaloyl-1-deoxy-1-(phenylsulfinyl)-β-L-glucopyranose (L-45)

By the method above, but beginning with L-glucose, the title compound is prepared.

7.23. 4-*O*-(2,3,4,6-tetra-*O*-pivaloyl-β-D-galactopyranosyl)-2,3,6-tri-*O*-pivaloyl-1-deoxy-1-phenylsulfinyl-β-D-glucopyranose (51)

To a solution of α-lactose monohydrate (**46**, 2.5 g, 6.9 mmol) in 45 mL of pyridine is added acetic anhydride (15 mL, 160 mmol) and DMAP (0.10 g, 0.82 mmol). The solution is stirred overnight and concentrated *in vacuo*. The residue is dissolved in 100 mL of CH_2Cl_2, washed with 1N HCl (3 x 50 mL), dried over Na_2SO_4, and concentrated to afford 4.7 g (100%) of octa-acetyl lactose **47** as the α anomer: R_f 0.50 (75% EtOAc/hexane); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 6.23 (d, J = 3.6 Hz, 1H, H-1), 5.44 (appt, J = 9.7 Hz, 1H), 5.34 (d, J = 2.6 Hz, 1H), 5.07-5.14 (m, 1H), 4.91-5.07 (m, 2H), 4.40-4.48 (m, 2H), 3.96-4.17 (m, 4H), 3.76-3.89 (m, 2H), 2.16 (s, 3H), 2.14 (s, 3H), 2.11 (s, 3H), 2.04 (s, 6H), 2.03 (s, 3H), 1.99 (s, 3H), 1.95 (s, 3H).

To a solution of octa-acetyl lactose **47** (4.7 g, 6.9 mmol) in 75 mL of CH_2Cl_2 is added thiophenol (1.42 mL, 13.9 mmol) followed by boron trifluoride diethyl ether complex (6.40 mL, 52.0 mmol). The reaction mixture is stirred at room temperature for 7 h and is then concentrated to half its volume by passing N_2 over it for 3 h. The reaction is stirred an additional 16 h at room temperature and then quenched by pouring slowly into 150 mL of saturated aqueous $NaHCO_3$ and stirring for 5 min. The product is extracted with CH_2Cl_2 (3 x 75 mL), dried over Na_2SO_4 and concentrated *in vacuo* to an oil which is purified by flash chromotography to give 4.1 g (81%) of the phenyl thioglycoside of hepta-acetyl lactose **48** as a 5:1 (β:α) mixture of anomers: R_f 0.34 (50% EtOAc/hexane); ^{1}H NMR ($CDCl_3$, 270 MHz) β-anomer, δ 7.46-7.49 (m, 2H), 7.26-7.32 (m, 3H), 5.34 (d, J = 3.3 Hz, 1H), 5.22 (appt, J = 9.1 Hz, 1H), 5.07-5.14 (m, 1H), 4.87-4.97 (m, 2H), 4.67 (d, J = 9.9 Hz, 1H), 4.45-4.56 (m, 2H), 4.03-4.16 (m, 3H), 3.86 (appt, J =

6.8 Hz, 1H), 3.72-3.79 (m, 1H), 3.61-3.67 (m, 1H), 2.15 (s, 3H), 2.11 (s, 3H), 2.09 (s, 3H), 2.05 (s, 6H), 2.04 (s, 3H), 1.97 (s, 3H).

To a solution of acetylated phenyl thioglycoside **48** (4.1 g, 5.6 mmol) in 80 mL of methanol is added sodium methoxide (0.690 g, 12.8 mmol). The solution is stirred for 2 h at room temperature. Amberlite resin is added to the reaction mixture and stirred for five min. The neutralized reaction mixture is filtered through Celite and concentrated *in vacuo*. The lactose phenyl thioglycoside **49** is taken on to the next step without further purification.

Lactose phenyl thioglycoside **49** is dissolved in 80 mL of pyridine, and pivaloyl chloride (20 mL, 170 mmol) and DMAP (0.10 g, 0.82 mmol) are added. The reaction mixture is heated to 110 °C for 24 h. Additional pivaloyl chloride (5 mL, 40 mmol) and DMAP (0.10 g, 0.82 mmol) are added. The reaction is heated for another 24 h at 110 °C. The reaction is allowed to cool, poured into 50 mL of methanol, and stirred for 30 min. EtOAc (500 mL) is added, and the organic layer is washed with 1N HCl (6 X 100 mL) and saturated NaCl (3 X 100 mL), dried over Na_2SO_4, and concentrated *in vacuo*. The residue is purified by flash chromatography (10% EtOAc/hexane) to give 3.3 g (57%) of the phenyl thioglycoside of hepta-pivaloyl lactose **50** as a white foam, a 5:1 (β:α) mixture of anomers: R_f 0.23 (10% EtOAc/hexane); ^{1}H NMR ($CDCl_3$, 300 MHz) β-anomer, δ 7.45-7.49 (m, 2H), 7.26-7.31 (m, 3H), 5.40 (d, *J* = 2.6 Hz, 1H), 5.24 (appt, *J* = 9.4 Hz, 1H), 5.08-5.15 (m, 1H), 4.94-5.02 (m, 1H), 4.85 (appt, *J* = 9.6 Hz, 1H), 4.70 (d, *J* = 10.2 Hz, 1H), 4.49-4.59 (m, 2H), 3.82-4.24 (m, 5H), 3.55-3.61 (m, 1H), 1.27 (s, 9H), 1.22 (s, 9H), 1.21 (s, 9H), 1.19 (s, 9H), 1.18 (s, 9H), 1.13 (s, 9H), 1.09 (s, 9H).

To a solution of hepta-pivaloyl lactose phenyl thioglycoside **50** (2.0 g, 1.9 mmol) in 60 mL of CH_2Cl_2

at -78 °C is added 65% *m*-CPBA (0.546 g, 2.02 mmol). The reaction mixture is allowed to warm to -15 °C and quenched with methyl sulfide (0.3 mL, 4 mmol). The reaction mixture is then diluted with 100 mL of saturated $NaHCO_3$ and extracted with CH_2Cl_2 (2 X 100 mL). The organic layers are combined, dried over Na_2SO_4, and concentrated *in vacuo*. The product is purified by flash chromatography (25% EtOAc/hexane) to afford 2.0 g (100%) of the title compound **51** as a mixture of diastereomers: R_f 0.19 (20% EtOAc/hexane).

7.24. 4-*O*-(2,3,4,6-tetra-*O*-pivaloyl-α-D-glucopyranosyl)-2,3,6-tri-*O*-pivaloyl-1-deoxy-1-phenylsulfinyl-β-D-glucopyranose (53)

By the method above, but beginning with maltose monohydrate (**52**), the title compound is obtained as a mixture of diastereomers, a white foam: R_f 0.31 (20% EtOAc/hexane).

7.25. 4-[(3-*O*-acetyl-2-azido-4,6-O-benzylidine-1,2-dideoxy-α-D-glucosyl)thio]phenoxyacetic acid (64)

To acetic anhydride (100 mL) is added mannose (0.1 g) and concentrated perchloric acid (8 drops). The solution is heated to 30°C and mannose (24.5 g, 136 mmol) is added in small portions over a period of 2 h while the reaction temperature is kept between 40-45 °C. The reaction mixture is allowed to cool and then stirred for 3 h at room temperature to produce crude mannose pentaacetate **54**: R_f 0.31 (50% EtOAc/hexane).

The reaction mixture is cooled at 10 °C and phosphorous tribromide (21.0 mL, 220 mmol) is added. To this solution, water (11 mL, 610 mmol) is added dropwise so that the internal temperature of the reaction mixture is maintained at 20-25 °C. After 30 minutes, the addition is complete, and the reaction mixture is stirred at room temperature for 1.5 h.

The reaction mixture is cooled to 5 °C and a solution of sodium acetate trihydrate (74.4 g, 547 mmol) in water (100 mL) is added dropwise over 30 minutes. Initially, the reaction is exothermic until approximately one third of the aqueous sodium acetate solution had been added. During the course of the addition, the temperature of the reaction mixture is maintained at 20-25 °C and then stirred at room temperature for 20 minutes. The reaction mixture is poured onto ice and extracted with $CHCl_3$ (3 x 120 mL), washed with water (300 mL), saturated $NaHCO_3$ (300 mL), dried over $MgSO_4$, filtered and concentrated to a yellow oil. The product is recrystallized from diethyl ether (200 mL) to afford 11.6 g (24%) of 1,3,4,6-tetra-*O*-acetyl-β-D-mannopyranose **55** as a white solid: R_f 0.39 (33% hexane/EtOAc); ^{1}H NMR ($CDCl_3$, 270 MHz) d 5.80 (t, *J* = 0.99 Hz, 1H, H-1), 5.39 (app t, *J* = 9.8 Hz, 1H, H-4), 5.05 (ddd, *J* = 9.8, 3.0, 0.99 Hz, 1H, H-3), 4.30 (ddd, *J* = 12.4, 4.9, 0.66, 1H, H-6), 4.20 (br s, 1H, H-2), 4.12 (dd, *J* = 12.5, 2.3 Hz, 1H, H-6), 3.78 (dddd, *J* = 9.7, 4.9, 2.3, 0.99 Hz, 1H, H-5), 2.18 (s, 3H), 2.12 (s, 3H), 2.05 (s, 3H), 2.10 (s, 3H).

To a solution of mannose tetraacetate **55** (1.50 g. 4.30 mmol) in 46 mL of CH_2Cl_2 is added pyridine (1.0 mL, 13 mmol). The solution is cooled to -25 °C and trifluoromethanesulfonic anhydride (1.8 mL, 2.98 g, 10.5 mmol) is added dropwise. The reaction mixture is stirred for 45 min at -25 °C and then diluted with 50 mL of CH_2Cl_2, washed with H_2O (100 mL), $NaHCO_3$ (100 mL), saturated NaCl (100 mL), dried over $MgSO_4$, filtered, and concentrated to afford an orange gel. The product is recrystallized from diethyl ether (25 mL) to afford 1.59 g (77%) of 1,3,4,6-tetra-*O*-acetyl-2-*O*-trifluoromethanesulfonyl-β-D-mannopyranose **56** as a white solid: R_f 0.49 (50% EtOAc/hexane); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.92 (s, 1H, H-1), 5.15-5.34 (m, 3H), 4.15-4.28 (m, 2H), 3.81-3.87

(m, 1H, H-5), 2.08 (s, 3H), 2.10 (s, 3H), 2.12 (s, 3H), 2.16 (s,3H).

To a solution of mannose triflate **56** (1.28 g, 2.66 mmol) in 14 mL of DMF is added sodium azide (0.69 g, 10.5 mmol). The reaction mixture is heated at 40 °C for 1.75 h, cooled to room temperature, diluted with 100 mL of CH_2Cl_2, washed with H_2O (100 mL), saturated NaCl (100 mL), dried over $MgSO_4$, filtered, and concentrated to afford a yellow oil. The product is purified by flash chromatography (33% EtOAc/hexane) to afford 0.80 g (81%) of 2-azido-2-deoxy-1,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose **57**: R_f 0.31 (33% EtOAc/hexane) δ 5.55 (d, *J* = 8.6 Hz, 1H, H-1), 5.01-5.15 (m, 2H, H-3, H-4), 4.31 (dd, *J* = 12.7, 4.5 Hz, 1H, H-6), 4.08 (d, *J* = 12.5 Hz, 1H, H-6), 3.77-3.84 (m, 1H, H-5), 3.69 (app t, *J* = 9.1 Hz, 1H, H-2), 2.10 (s, 3H), 2.08 (s, 3H), 2.05 (s, 3H), 2.03 (s, 3H).

To a solution of 2-azido glucose tetraacetate **57** (0.222 g, 0.59 mmol) in 6.6 mL of CH_2Cl_2 is added 4-hydroxythiophenol (0.146 g, 1.15 mmol) followed by boron trifluoride diethyl ether complex (15.9 mL, 18.4 g. 141 mmol). The reaction mixture is heated at 45 °C for 12 h and then quenched by the addition of 2 mL of H_2O. The reaction mixture is diluted with 25 mL of CH_2Cl_2, washed with H_2O (10 mL), saturated NaCl (10 mL), dried over Na_2SO_4, filtered, and concentrated to afford 0.232 g of crude 2-azido-1,2-dideoxy-1-(4-hydroxyphenylthio)-3,4,6-tri-*O*-acetyl-α,β-D-glucopyranose **58** as a yellow oil, a mixture of anomers which is then taken on to the next step without further purfication.

To a solution of the thioglycoside anomers **58** in 5.2 mL of methanol is added K_2CO_3 (0.146 g, 1.06 mmol). The reaction mixture is stirred at room temperature for 10 min. Amberlite resin (acid form) is added to the reaction mixture and stirred for an additional 15 min. The neutralized mixture is then

filtered through Celite, washed several times with methanol, and concentrated to afford 0.512 g of 2-azido-1,2-dideoxy-1-(4-hydroxyphenylthio)-α,β-D-glucopyranose **59**. The product is purified by flash chromatography over silica gel (10% MeOH in CH_2Cl_2) to afford 0.028 g (15% from **57**) as a 2:1 (α, β) mixture of anomers: R_f (α-anomer) 0.33 (10% MeOH/CH_2Cl_2); R_f (β-anomer) 0.26 (10% MeOH/CH_2Cl_2); ^{1}H NMR (d_6-acetone, 270 MHz, mixture of anomers) δ 8.69 (br s, 1H), 8.63 (br s, 1H), 7.45 (d, J = 8.9 Hz, 2H), 7.40 (d, J = 8.6 Hz, 2H), 6.84 (d, J = 8.6 Hz, 2H), 6.81 (d, J = 8.6 Hz, 2H), 5.36 (d, J = 4.6 Hz, 1H), 4.85-4.89 (m, 2H), 4.55 (d, J = 5.3 Hz, 1H), 4.40 (d, J = 9.2 Hz, 1H), 4.43-4.45 (m, 2H), 4.11-4.18 (m, 1H), 3.42-3.88 (m, 7H), 3.27-3.37 (m, 4H), 3.08 (app t, J = 9.7 Hz, 1H).

To a solution of mixed anomers **59** (2.10 g, 6.70 mmol) in 85 mL of THF is added benzaldehyde dimethyl acetal (3.0 mL, 3.10 g, 20.1 mmol) and camphorsulfonic acid (0.31 g, 1.34 mmol). The reaction mixture is stirred at 62 °C for 7 h and then at room temperature for 10 h, cooled and concentrated to afford a brown oil. The product is purified by flash chromatography on silica gel (20% EtOAc/hexane) to afford 1.16 g (43%) of 2-azido-4,6-*O*-benzylidene-1,2-dideoxy-1-(4-hydroxyphenylthio)-α,β-D-glucopyranose **60** as a mixture of anomers. The anomers are separable by flash chromatography (20% EtOAc/hexane): R_f (α-anomer) 0.31 (40% EtOAc/hexane); R_f (β-anomer) 0.42 (40% EtOAc/hexane); ^{1}H NMR ($CDCl_3$, 270 MHz) α-anomer, δ 7.33-7.55 (m, 7H), 6.84 (d, J = 8.9 Hz, 2H), 5.64 (s, 1H), 5.49 (d, J = 4.6 Hz, 1H, H-1), 4.32 (td, J = 9.8, 4.9 Hz, 1H, H-5), 4.16 (dd, J = 10.2, 4.9 Hz, 1H, H-6), 3.92-4.03 (m, 2H), 3.77 (app t, J = 10.2 Hz, 1H, H-6), 3.63 (app t, J = 9.3 Hz, 1H, H-4). The mixed anomers are carried on to the following step without separation.

To a solution of 4,6-benzylidene protected

glucose **60** (1.04 g, 2.58 mmol) and 2-(trimethylsilyl)ethyl 2-bromoacetate (1.27 g, 5.16 mmol) in 25 mL of DMF is added K_2CO_3 (0.35 g, 2.58 mmol). The reaction mixture is heated at 45 °C for 12 h, cooled, diluted with 50 mL of CH_2Cl_2, washed with saturated $NaHCO_3$ (75 mL) and then extracted with CH_2Cl_2 (2 x 50 mL), washed with H_2O (75 mL), saturated NaCl (75 mL), dried over Na_2SO_4, filtered and concentrated to afford 5.40 g of the anomers **61** and **62** of 2-(trimethylsilyl)ethyl 2-{4-[(2-azido-4,6-*O*-benzylidene-1,2-dideoxy-D-glucopyranosyl)thio]-phenoxy}acetate. The anomers are separated by flash chromatography (20% EtOAc/hexane) to afford 0.811 g (56%) of α-anomer **61**: R_f 0.59 (40% EtOAc/hexane), and 0.496 g (34%) of β-anomer **62**: R_f 0.70 (40% EtOAc/hexane); ^1H NMR ($CDCl_3$, 300 MHz) α-anomer, d 7.28-7.55 (m, 7H), 7.84 (d, *J* = 8.9 Hz, 2H), 5.50 (s, 1H), 5.36 (d, *J* = 5.5 Hz, 1H, H-1), 4.54 (s, 2H), 4.34 (td, *J* = 9.9, 4.7 Hz, 1H, H-5), 4.25 (m, 2H), 4.18 (dd, *J* = 10.4, 4.9 Hz, 1H, H-6), 3.97 (ddd, *J* = 9.9, 9.9, 1.8 Hz, 1H, H-3), 3.83 (dd, *J* = 9.8, 5.5 Hz, 1H, H-2), 3.69 (app t, *J* = 10.2 Hz, 1H, H-6), 3.51 (app t, *J* = 9.3 Hz, 1H, H-4), 2.90 (br s, 1H), 1.0 (m, 2H), 0.50 (s, 9H); ^1H NMR ($CDCl_3$, 270 MHz) β-anomer, δ 7.33-7.55 (m, 7H), 6.88 (d, *J* = 8.9 Hz, 2H), 5.52 (s, 1H), 4.62 (s, 2H), 4.43 (d, *J* = 10.2 Hz, 1H, H-1), 4.29-4.39 (m, 1H, H-6), 4.32 (m, 2H), 3.72-3.80 (m, 2H), 3.43-3.48 (m, 2H), 3.30 (app t, *J* = 9.7 Hz, 1H, H-2), 1.02-1.07 (m, 2H), 0.50 (s, 9H).

To a solution of **61** (0.848 g, 1.51 mmol) in 15 mL of CH_2Cl_2 is added acetic anhydride (0.43 mL, 0.46 g, 4.54 mmol), triethylamine (0.63 mL, 0.46 g, 4.54 mmol), and DMAP (0.187 g, 1.51 mmol). The reaction is stirred at room temperature for 5 min and then diluted with 10 mL of CH_2Cl_2, washed with $NaHCO_3$ (15 mL), extracted with CH_2Cl_2 (2 x 15 mL), washed with saturated NaCl (20 mL), dried over Na_2SO_4, filtered and concentrated to afford 1.07 g of a yellow-orange

oil. The product is purified by flash chromatography (17% EtOAc/hexane) to afford 0.660 g (73%) of 2-(trimethylsilyl)ethyl 2-{4-[(3-*O*-acetyl-2-azido-4,6-*O*-benzylidene-1,2-dideoxy-α-D-glucopyranosyl)thio]-phenoxy}acetate **63**: R_f 0.18 (17% EtOAc/hexane); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 7.29-7.43 (m, 7H), 6.81 (d, *J* = 8.9 Hz, 2H), 5.39-5.46 (m, 3H), 4.44 (td, *J* = 9.9, 4.9 Hz, 1H, H-5), 4.24 (m, 2H), 4.18 (dd, *J* = 10.2, 4.9 Hz, 1H, H-6), 3.96 (dd, *J* = 10.2, 5.6 Hz, 1H, H-2), 3.71 (app t, *J* = 10.2 Hz, 1H, H-6), 3.60 (app t, *J* = 9.6 Hz, 1H, H-4), 2.11 (s, 3H), 1.0 (m, 2H), 0.87 (s, 9H).

To a solution of **63** (0.044 g, 0.073 mmol) in 0.4 mL of THF is added tetra-*n*-butylammonium fluoride solution (1.0 M in THF, 0.36 mL, 0.36 mmol). The reaction mixture is stirred for 20 min at room temperature and diluted with 3 mL of CH_2Cl_2, washed with 5% HCl (5 mL), extracted with CH_2Cl_2 (2 x 2 mL), washed with saturated NaCl (5 mL), dried over Na_2SO_4, filtered, and concentrated to afford 0.067 g of a clear oil. The product is purified by flash chromatography (2% MeOH/$CHCl_3$) to afford 0.035 g (95%) of 2-{4-[(3-*O*-acetyl-2-azido-4,6-*O*-benzylidene-1,2-dideoxy-α-D-glucopyranosyl)thio]phenoxy}acetic acid **64** as a white solid: R_f 0.39 (5% MeOH/$CHCl_3$); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 7.35-7.49 (m, 7H), 6.90 (d, *J* = 8.9 Hz, 2H), 5.45-5.52 (m, 3H), 4.69 (s, 2H), 4.48 (td, *J* = 9.8, 5.2 Hz, 1H, H-5), 4.24 (dd, *J* = 10.4, 4.7 Hz, 1H, H-6), 4.03 (dd, *J* = 10.2, 5.6 Hz, 1H, H-2), 3.78 (app t, *J* = 10.3 Hz, 1H, H-6). 3.67 (app t, *J* = 9.6 Hz, 1H, H-4), 2.15 (s, 3H).

7.26. 2-{4-[(3-*O*-acetyl-2-azido-4,6-O-benzylidine-1,2-dideoxy-β-D-glucosyl)thio]phenoxy}-acetic acid (66)

To a solution of **62** (0.496 g, 0.886 mmol) in 10 mL of CH_2Cl_2 is added acetic anhydride (0.25 mL, 0.27 g, 2.65 mmol), triethylamine (0.37 mL, 0.27 g,

2.65 mmol), and DMAP (0.108 g, 0.886 mmol). The reaction is stirred at room temperature for 20 min and then diluted with 3 mL of CH_2Cl_2, washed with $NaHCO_3$ (10 mL), extracted with CH_2Cl_2 (2 x 3 mL), washed with saturated NaCl (5 mL), dried over Na_2SO_4, filtered and concentrated to afford 0.612 g of a yellow oil. The product is purified by flash chromatography (17% EtOAc/hexane) to afford 0.438 g (82%) of 2-(trimethylsilyl)ethyl 2-{4-[(3-*O*-acetyl-2-azido-4,6-*O*-benzylidene-1,2-dideoxy-β-D-glucopyranosyl)thio]phenoxy}acetate **65**: R_f 0.56 (40% EtOAc/hexane); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.27-7.55 (m, 7H), 6.85 (d, *J* = 8.8 Hz, 2H), 5.41 (s, 1H), 5.17 (app t, *J* = 9.4Hz, 1H, H-3), 4.57 (s, 2H), 4.43 (d, *J* = 10.3 Hz, 1H, H-1), 4.30-4.34 (m, 1H), 4.26 (m, 2H), 3.70 (ddd, *J* = 10.3, 9.9, 2.7 Hz, 1H, H-5), 3.40-3.49 (m, 2H, H-4, H-6), 3.29 (app t, *J* = 9.7 Hz, 1H, H-2), 2.24 (s, 3H), 0.99 (m, 2H), 0.01 (s, 9H). To a solution of **65** (0.425 g, 0.706 mmol) in 7 mL of THF is added tetra-*n*-butylammonium fluoride solution (1.0 M in THF, 3.5 mL, 3.53 mmol). The reaction mixture is stirred for 5 min at room temperature and diluted with 10 mL of CH_2Cl_2, washed with 5% HCl (10 mL), extracted with CH_2Cl_2 (2 x 5 mL), washed with saturated NaCl (10 mL), dried over Na_2SO_4, filtered, and concentrated to afford 0.65 g of a clear oil. The product is purified by flash chromatography (gradient elution: 50% EtOAc/hexane, 100% EtOAc, 20% $MeOH/CHCl_3$) to afford 0.30 g (85%) of the title compound **66** as a white solid: R_f 0.48 (5% $MeOH/CHCl_3$); ^{1}H NMR (C_6D_6, 300 MHz) δ 7.34-7.62 (m, 7H), 7.05 (d, *J* = 8.8 Hz, 2H), 5.67 (s, 1H), 5.34 (app t, *J* = 9.3 Hz, 1H, H-3), 4.92 (d, *J* = 10.3 Hz, 1H, H-1), 4.77 (s, 2H), 4.36 (dd, *J* = 9.3, 3.5 Hz, 1H), 3.70-3.90 (m, 3H), 3.52 (app t, *J* = 9.9 Hz, 1H, H-2), 2.05 (s, 3H).

7.27. 2-{4-[(3-*O*-acetyl-2-azido-4,6-*O*-benzylidene-1,2-dideoxy-β-D-galactopyranosyl)thio]-phenoxy}acetic acid (76)

To a solution of tri-*O*-acetyl-D-galactal **67** (25.0 g, 91.8 mmol) in 1000 mL of distilled CH_3CN at -20 °C is added sodium azide (8.96 g, 138 mmol), followed by ceric ammonium nitrate (151 g, 276 mmol). The reaction suspension is stirred vigorously at -15 to -20 °C for 24 h and then filtered through Celite. The filtrate is diluted with 1000 mL of ice water and extracted with CH_2Cl_2 (3 x 200 mL). The combined organic layers are dried over Na_2SO_4, concentrated, and purified by flash chromatography (10% EtOAc/hexane) to give 17.5 g (51%) of 2-azido-2-deoxy-1-*O*-nitro-3,4,6-tri-*O*-acetyl-α,β-D-galactopyranose **68** as a mixture of anomers: R_f 0.26 (25% EtOAc/hexane); ^{1}H NMR ($CDCl_3$, 270 MHz, mixture of anomers) δ 6.32 (d, *J* = 4.0 Hz, 1 H, H-1α), 5.55 (d, *J* = 8.9 Hz, 1 H, H-1β), 5.48 (d, *J* = 3.0 Hz, 1 H, H-4α), 5.37 (d, *J* = 3.0 Hz, 1 H, H-4β), 5.23 (dd, *J* = 11.6, 3.3 Hz, 1 H, H-3α), 4.93 (dd, *J* = 10.6, 3.3 Hz, 1 H, H-3β), 4.35 (t, *J* = 6.6 Hz, 1 H, H-5α), 3.9-4.2 (m, 6 H, H-2α, H-5β, H-6β, H-6α), 3.81 (dd, *J* = 10.6, 8.9 Hz, 1 H, H-2β), 1.95-2.25 (6s,18 H).

To a solution of nitrate ester **68** (17.5 g, 46.5 mmol) in 500 mL of glacial acetic acid is added sodium acetate (7.63 g, 93.0 mmol). The solution is stirred at 100 °C for 3 h and then allowed to cool to room temperature. The reaction mixture is diluted with 1000 mL of ice water and extracted with CH_2Cl_2 (2 x 200 mL). The organic layers are combined and washed with ice water (2 x 400 mL), saturated $NaHCO_3$ (400 mL), saturated NaCl (400 mL), dried over Na_2SO_4, filtered and concentrated. The crude product is crystallized from hexane/EtOAc to give 7.2 g of 2-azido-2-deoxy-1,3,4,6-tetra-*O*-acetyl-α,β-D-galactopyranose **69** (mixed anomers). The mother liquors from the crystallization are concentrated and

purified by flash chromatography (25% EtOAc/hexane) to give an additional 7.0 g of the product (14.2 g, 81.7% in total) as a mixture of anomers: R_f 0.3 (30% EtOAc/hexane); ^{1}H NMR ($CDCl_3$, 270 MHz, mixture of anomers) δ 6.26 (d, J = 3.6 Hz, 1 H, H-1α), 5.50 (d, J = 8.6 Hz, 1 H, H-1β), 5.42 (d, J = 3.0 Hz, 1 H, H-4α), 5.32 (d, J = 3.0 Hz, 1 H, H-4β), 5.25 (dd, J = 11.2, 3.0 Hz, 1 H, H-3α), 4.85 (dd, J = 10.6, 3.3 Hz, 1 H, H-3β), 4.23 (t, J = 6.6 Hz, 1 H, H-5α), 3.95-4.10 (m, 5 H, H-6α, H-5β, H-6β), 3.88 (dd, J = 11.2, 3.6 Hz, 1 H, H-2α), 3.78 (dd, J = 10.9, 8.6 Hz, 1 H, H-2β), 1.90-2.20 (6s, 24 H, OAc).

To a solution of **69** (1.16 g, 2.95 mmol) in 100 mL of CH_2Cl_2 is added 4-hydroxythiophenol (0.90 g, 5.9 mmol), followed by boron trifluoride diethyl ether complex (1.5 mL, 11.8 mmol). The mixture is refluxed at 50 °C for 48 h and then quenched by the addition of 200 mL of H_2O. The reaction mixture is diluted with 200 mL of CH_2Cl_2. The organic layer is washed with H_2O (200 mL), saturated $NaHCO_3$ (2 x 200 mL), dried over Na_2SO_4, filtered, and concentrated to give 2-azido-1,2-dideoxy-1-(4-hydroxyphenylthio)-3,4,6-tri-*O*-acetyl-α,β-D-galactopyranose **70** as a brown oil, R_f 0.13 (30% EtOAc/hexane, which is taken on to the next reaction without further purification. A purified sample of the β-anomer had ^{1}H NMR ($CDCl_3$, 270 MHz) δ 7.51 (d, J = 8.9 Hz, 2 H, ArH), 6.82 (d, J = 8.9 Hz, 2 H, ArH), 5.33 (dd, J = 3.3, 1.0 Hz, 1 H, H-4), 5.08 (s, 1 H, ArOH), 4.85 (dd, J = 10.2, 3.3 Hz, 1 H, H-3), 4.24 (d, J = 10.2 Hz, 1 H, H-1), 4.17 (dd, J = 11.2, 6.6 Hz, 1 H, H-6), 4.09 (dd, J = 11.2, 6.6 Hz, 1 H, H-6'), 3.85 (dt, J = 6.6, 1.0 Hz, 1 H, H-5), 3.57 (t, J = 10.2 Hz, 1 H, H-2), 2.08 (s, 3 H, OAc), 2.04 (s, 3 H, OAc), 2.03 (s, 3 H, OAc).

To a solution of **70** in 50 mL of MeOH is added K_2CO_3 until pH paper indicated the solution to be basic (pH 11). The reaction mixture is stirred at room temperature for 15 min and then neutralized with

Amberlite resin (acid form). The resin is removed by filtration and washed with MeOH (2 x 50 mL). The filtrates are concentrated and purified by flash chromatography (70% EtOAc/hexane) to give 0.65 g (63%) of 2-azido-1,2-dideoxy-1-(4-hydroxyphenylthio)-α,β-D-galactopyranose **71**: R_f 0.19 (100% EtOAc).

To a solution of **71** (1.20 g, 3.65 mmol) in 100 mL of DMF is added benzaldehyde dimethyl acetal (1.65 mL, 10.9 mmol) and *p*-toluenesulfonic acid (0.14 g, 0.73 mmol). The reaction is stirred at room temperature for 8 h and then neutralized with saturated $NaHCO_3$. The reaction mixture is diluted with 200 mL of EtOAc, washed with saturated NaCl (3 x 200 mL), dried over Na_2SO_4, filtered, concentrated and purified by flash chromatography (35% EtOAc/hexane) to give 1.30 g (89%) of 2-azido-4,6-*O*-benzylidene-1,2-dideoxy-1-(4-hydroxyphenylthio)-α,β-D-galactopyranose **72**. The mixed anomers are used directly for the next step, although the anomers are separable by flash chromatography (35% EtOAc/hexane): R_f (α-anomer) 0.28 (40% EtOAc/hexane); R_f (β-anomer) 0.11 (40% EtOAc/hexane); ^{1}H NMR ($CDCl_3$, 270 MHz) β-anomer, δ7.61 (d, J = 8.6 Hz, 2 H, ArH), 7.37-7.39 (m, 5 H, ArH), 6.76 (d, J = 8.6 Hz, 2 H, ArH), 5.51 (s, 1 H, CH), 4.96 (s, 1 H, ArOH), 4.38 (dd, J = 12.5, 1.7 Hz, 1 H, H-6), 4.32 (d, J = 9.6 Hz, 1 H, H-1), 4.17 (d, J = 3.6 Hz, 1 H, H-4), 4.02 (dd, J = 12.5, 1.7 Hz, 1 H, H-6'), 3.65 (dt, J = 9.6, 3.6 Hz, 1 H, H-3), 3.51 (d, J = 1.3 Hz, 1 H, H-5), 3.45 (t, J = 9.6 Hz, 1 H, H-2), 2.50 (d, J = 9.9 Hz, 1 H, OH-3); α-anomer, δ7.36-7.50 (m, 7 H, ArH), 6.77-6.80 (m, 2 H, ArH), 5.61 (s, 1 H, CH), 5.57 (d, J = 5.3 Hz, 1 H, H-1), 4.82 (s, 1 H, ArOH), 4.34 (dd, J = 3.6, 1.0 Hz, 1 H, H-4), 4.30 (d, J = 1.0 Hz, 1 H, H-5), 4.11-4.30 (m, 3 H, H-6, H-6'), 4.17 (dd, J = 10.2, 5.3 Hz, 1 H, H-2), 4.00 (dt, J =10.2, 3.6 Hz, 1 H, H-3), 2.52 (d, J = 10.2 Hz, 1 H, OH-3).

To a solution of **72** (1.70 g, 4.20 mmol) in 60 mL

of DMF is added K_2CO_3 (0.58 g, 4.2 mmol) and 2-(trimethylsilyl)ethyl bromoacetate (2.0 g, 8.5 mmol). The reaction mixture is stirred at 50-60 °C for 3 h and then allowed to cool to room temperature. The reaction mixture is diluted with EtOAc (150 mL), washed with H_2O (3 x 80 mL) and saturated NaCl (80 mL), dried over Na_2SO_4, concentrated and purified by flash chromatography (45% EtOAc/hexane) to give 1.60 g (68%) of 2-(trimethylsilyl)ethyl 2-{4-[(2-azido-4,6-*O*-benzylidene-1,2-dideoxy-α,β-D-galactopyranosyl)thio]phenoxy}acetate **73** as a mixture of anomers. The mixed anomers are used directly for the next step, but could be separated by flash chromatography (25% EtOAc/hexane to elute the α-anomer, 45% EtOAc/hexane to elute the β-anomer): R_f (α-anomer) 0.15 (25% EtOAc/hexane); R_f (β-anomer) 0.12 (35% EtOAc/hexane); ^{1}H NMR ($CDCl_3$, 270 MHz) α-anomer, δ 7.38-7.48 (m, 7 H, ArH), 6.86 (d, *J* = 8.8 Hz, 2 H, ArH), 5.61 (s, 1 H, CH), 5.59 (d, *J* = 5.5 Hz, 1 H, H-1), 4.59 (s, 2 H, OCH_2CO), 3.96-4.33 (m, 8 H, H-2, H-3, H-4, H-5, H-6, H-6', $COOCH_2$), 1.06 (t, *J* = 8.8 Hz, 2 H, CH_2TMS), 0.05 (s, 9 H, $SiMe_3$); β-anomer, δ 7.66 (d, *J* = 8.8 Hz, 2 H, ArH), 7.39 (s, 5 H, ArH), 6.82 (d, *J* = 9.2 Hz, 2 H, ArH), 5.52 (s, 1 H, CH), 4.55 (s, 2 H, OCH_2CO), 4.28-4.39 (m, 5 H, H-1, H-3, H-6, $COOCH_2$), 4.16 (d, *J* = 3.3 Hz, 1 H, H-4), 4.02 (d, *J* = 12.5 Hz, 1 H, H-6'), 3.49 (s, 1 H, H-5), 3.45 (t, *J* = 9.9 Hz, 1 H, H-2), 1.04 (t, *J* = 8.8 Hz, 2 H, CH_2TMS), 0.05 (s, 9 H, $SiMe_3$).

To a solution of **73** (1.60 g, 2.86 mmol) in 50 mL of CH_2Cl_2 is added Et_3N (0.8 mL, 2.86 mmol), acetic anhydride (0.6 mL, 5.72 mmol) and DMAP (0.35 g, 2.86 mmol). The reaction mixture is stirred at room temperature for 15 min. The reaction is diluted with 100 mL of CH_2Cl_2 and washed with saturated $NaHCO_3$ (80 mL), dried over Na_2SO_4, filtered, concentrated and purified by flash chromatography to give a combined yield of 1.31 g (76.4%) of the anomers of 2-

(trimethylsilyl)ethyl 2-{4-[(3-*O*-acetyl-2-azido-4,6-*O*-benzylidene-1,2-dideoxy-α,β-D-galactopyranosyl)-thio]phenoxy}acetate (15% EtOAc/hexane to elute the α-anomer **75**, 35% EtOAc/hexane to elute the β-anomer **74**).

74: R_f 0.31 (30% EtOAc/hexane); ^{1}H NMR ($CDCl_3$, 270 MHz), δ 7.65 (d, J = 8.8 Hz, 2 H, ArH), 7.39 (s, 5 H, ArH), 6.77 (d, J = 8.8 Hz, 2 H, ArH), 5.47 (s, 1 H, CH), 4.80 (dd, J = 10.6, 3.3 Hz, 1 H, H-3), 4.52 (s, 2 H, OCH_2CO), 4.42 (d, J = 9.9 Hz, 1 H, H-1), 4.36 (d, J = 12.1 Hz, 1 H, H-6), 4.31 (t, J = 8.8, 8.4 Hz, 2 H, $COOCH_2$), 4.29-4.31 (m, 1 H, H-4), 4.00 (d, J = 12.8 Hz, 1 H, H-6'), 3.76 (t, J = 10.6, 9.9 Hz, 1 H, H-2), 3.54 (s, 1 H, H-5), 2.10 (s, 3 H, OAc), 1.04 (t, J = 8.8, 8.4 Hz, 2 H, CH_2TMS), 0.06 (s, 9 H, $SiMe_3$);

75: R_f 0.38 (25% EtOAc/hexane); ^{1}H NMR ($CDCl_3$, 270 MHz), δ 7.33-7.49 (m, 5 H, ArH), 7.39 (d, J = 8.9 Hz, 2 H, ArH), 6.83 (d, J = 8.9 Hz, 2 H, ArH), 5.61 (d, J = 5.3 Hz, 1 H, H-1), 5.52 (s, 1 H, CH), 5.01 (dd, J = 11.2, 3.6 Hz, 1 H, H-3), 4.56 (s, 2 H, OCH_2CO), 4.49-4.55 (m, 2 H, H-2, H-4), 4.28 (m, 2 H, $COOCH_2$), 4.24 (br s, 1 H, H-5), 4.18(dd, J = 12.5, 1.3 Hz, 1 H, H-6), 4.07 (dd, J = 12.5, 1.3 Hz, 1 H, H-6'), 2.14 (s, 3 H, OAc), 1.01 (m, 2 H, CH_2TMS), 0.03 (s, 9 H, $SiMe_3$); ^{13}C NMR ($CDCl_3$, 270 MHz), δ 170.4, 168.8, 158.0, 137.5, 134.3, 129.2, 128.3, 126.2, 124.9, 115.6, 100.9, 88.3, 73.3, 71.4, 69.2, 65.6, 64.0, 63.4, 57.9, 21.1, 17.5, -1.35.

To a solution of **74** (0.628 g, 1.04 mmol) in 10 mL of THF is added tetra-n-butylammonium fluoride (2.1 mL of 1.0 M solution in THF, 2.09 mmol). The reaction mixture is stirred at room temperature for 10 min and then neutralized with dilute HCl, concentrated and purified by flash chromatography (0.1% HOAc, 5% MeOH, in EtOAc) to give 0.486 g (93%) of the title compound **76**: R_f 0.14 (10% MeOH/EtOAc); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 7.64 (d, J = 8.6 Hz, 2 H,

ArH), 7.37 (s, 5 H, ArH), 6.73 (d, J = 8.9 Hz, 2 H, ArH), 5.45 (s, 1 H, CH), 4.81 (dd, J = 10.2, 3.0 Hz, 1 H, H-3), 4.53 (s, 2 H, OCH_2CO), 4.42 (d, J = 9.9 Hz, 1 H, H-1), 4.33 (d, J = 11.9 Hz, 1 H, H-6), 4.28 (d, J = 3.0 Hz, 1 H, H-4), 3.96 (d, J = 11.9 Hz, 1 H, H-6'), 3.75 (t, J = 10.2, 9.9 Hz, 1 H, H-2), 3.50 (s, 1 H, H-5), 2.08 (s, 3 H, OAc). ^{13}C NMR ($CDCl_3$, 270 MHz) δ 173.4, 170.7, 158.2, 137.6, 136.6, 129.3, 128.3, 126.4, 121.9, 115.3, 100.7, 85.3, 74.0, 72.7, 69.5, 69.2, 64.8, 58.3, 21.1.

7.28. 2-{4-[(3-*O*-acetyl-2-azido-4,6-*O*-benzylidene-1,2-dideoxy-α-D-galactopyranosyl)thio]-phenoxy}acetic acid (77)

The title compound is prepared from **75** by treatment with tetrabutylammonium fluoride, and purified by flash chromatography, as described above: R_f 0.14 (30% MeOH/EtOAc); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 7.44-7.48 (m, 2 H, ArH), 7.33-7.38 (m, 5 H, ArH), 6.80 (d, J = 8.6 Hz, 2 H, ArH), 5.61 (d, J = 4.9 Hz, 1 H, H-1), 5.49 (s, 1 H, CH), 4.48-4.56 (m, 4 H, H-2, H-4, OCH_2CO), 5.10 (dd, J = 10.9, 3.3 Hz, 1 H, H-3), 4.21 (s, 1 H, H-5), 4.14 (d, J = 12.9 Hz, 1 H, H-6), 4.04 (d, J = 12.2 Hz, 1 H, H-6'), 2.16 (s, 3 H, OAc). ^{13}C NMR ($CDCl_3$, 270 MHz) δ 173.9, 170.6, 157.7, 137.5, 134.5, 129.3, 128.3, 126.3, 125.2, 115.7, 100.9, 88.2, 73.3, 71.5, 69.2, 65.3, 63.5, 57.9, 21.1.

7.29. 2-{4-[(3-*O*-acetyl-4-azido-2,6-bis-*O*-(4-methoxybenzyl)-1,4-dideoxy-β-D-glucopyranosyl)thio]-phenoxy}acetic acid (88)

To a solution of galactose pentaacetate (3.30 g, 8.50 mmol) and 4-hydroxy-thiophenol (1.40 g, 11.1 mmol) in 50 mL of methylene chloride at -78 °C is added boron trifluoride diethyl ether complex (2.10 mL, 17.1 mmol). The solution is allowed to warm slowly to 0 °C and then stirred for an additional hour at 0 °C. The mixture is poured into saturated aqueous $NaHCO_3$ (200 mL) and extracted with CH_2Cl_2 (2 x

150 mL), dried over Na_2SO_4, concentrated, and purified by flash chromatography (50% EtOAc/hexane) to give 3.90 g (99%) of 1-deoxy-1-(4-hydroxyphenylthio)-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranose **78** as a white solid: R_f 0.3 (50% EtOAc/hexane); ^1H NMR ($CDCl_3$, 300 MHz) δ 7.42 (d, *J* = 8.6 Hz, 2H), 6.79 (d, *J* = 8.6 Hz, 2H), 5.78 (br s, 1H), 5.39 (d, *J* = 2.56 Hz, 1H, H-4), 5.15 (t, *J* = 9.9 Hz, 1H, H-2), 5.00 (dd, *J* = 3.3, 9.9 Hz, 1H, H-3), 4.56 (d, *J* = 9.9 Hz, 1H, H-1), 4.19 (dd, *J* = 6.6, 11.0 Hz, 1H), 4.09 (dd, *J* = 6.6, 11.0 Hz, 1H), 3.89 (t, *J* = 6.6 Hz, 1H, H-5), 2.12 (s, 3H, OAc), 2.09 (s, 3H, OAc), 2.05 (s, 3H, OAc), 1.97 (s, 3H, OAc).

To a solution of thioglycoside **78** (1.40 g, 3.10 mmol) in 15 mL of methylene chloride is added N,N-diisopropylethylamine (0.64 mL, 3.70 mmol), followed by 2-(trimethylsilyl)ethoxymethyl chloride (0.60 mL, 3.40 mmol). The mixture is stirred at room temperature for 2 h, poured into saturated NaCl (50 mL) and extracted with CH_2Cl_2 (2 x 50 mL), dried over Na_2SO_4, concentrated, and purified by flash chromatography (40% EtOAc/hexane) to give 1.60 g (89%) of 1-deoxy-1-{4-[2-(trimethylsilyl)ethoxymethoxy]phenylthio}-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranose **79** as a colorless oil: R_f 0.4 (33% EtOAc/hexane); ^1H NMR ($CDCl_3$, 270 MHz) δ 7.46 (d, *J* = 8.4 Hz, 2H), 6.98 (d, *J* = 8.4 Hz, 2H), 5.40 (d, *J* = 3.3 Hz, 1H, H-4), 5.22 (s, 2H, SEM), 5.20 (dd, *J* = 9.9, 9.9 Hz, 1H, H-2), 5.02 (dd, *J* = 3.3, 9.9 Hz, 1H, H-3), 4.58 (d, *J* = 9.9 Hz, 1H, H-1), 4.18 (dd, *J* = 6.6, 11.2 Hz, 1H), 4.10 (dd, *J* = 6.6, 11.2 Hz, 1H), 3.89 (dd, *J* = 6.6, 6.6 Hz, 1H, H-5), 3.75 (t, *J* = 8.4 Hz, 2H, SEM), 2.11 (s, 3H, OAc), 2.10 (s, 3H, OAc), 2.04 (s, 3H, OAc), 1.98 (s, 3H, OAc), 0.94 (t, *J* = 8.4 Hz, 2H, CH_2-TMS), 0.01 (s, 9H, $SiMe_3$).

To a solution of the SEM protected thioglycoside **79** (1.60 g, 2.73 mmol) in 15 mL of methanol is added

sodium methoxide (300 mg, 5.56 mmol). The mixture is stirred at room temperature for 12 h, then neutralized with Amberlite resin (acid form), filtered, concentrated and run through a short column of silica gel (10% MeOH/EtOAc) to provide crude 1-deoxy-1-{4-[2-(trimethylsilyl)ethoxymethoxy]phenylthio}-β-D-galactopyranose **80** as an oil: R_f 0.15 (EtOAc). The material is taken up in 10 mL of DMF, and dimethoxypropane (0.59 mL, 4.8 mmol), and *p*-toluenesulfonic acid hydrate (90 mg, 0.48 mmol) are added. The mixture is stirred at room temperature for 12 h, pyridine (0.04 mL, 0.48 mmol) is added, and the reaction mixture is concentrated and purified by flash chromatography (60% EtOAc/hexane) to give 0.5 g (41%) of 1-deoxy-3,4-*O*-isopropylidene-1-{4-[2-(trimethylsilyl)ethoxymethoxy]phenylthio}-β-D-galactopyranose **81** as a colorless oil: R_f 0.5 (67% EtOAc/hexane); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.48 (d, J = 8.6 Hz, 2H), 6.99 (d, J = 8.6 Hz, 2H), 5.21 (s, 2H, SEM), 4.35 (d, J = 10.2 Hz, 1H, H-1), 4.17 (dd, J = 1.7, 5.4 Hz, 1H), 4.09 (dd, J = 6.5 Hz, 1H), 3.94 (m, 1H), 3.85 (m, 2H), 3.73 (t, J = 8.1 Hz, 2H, SEM), 3.52 (m, 1H), 2.49 (d, J = 1.8 Hz, 1H), 2.17 (d, J = 9.1 Hz, 1H), 1.40 (s, 3H, Me), 1.33 (s, 3H, Me), 0.93 (t, J = 8.1 Hz, 2H, CH_2-TMS), 0.01 (s, 9H, $SiMe_3$).

To a solution of acetonide **81** (80 mg, 0.175 mmol) in 3 mL of DMF is added tetrabutylammonium iodide (194 mg, 0.52 mmol), 4-methoxybenzyl choride (0.19 mL, 1.40 mmol), and a 95% dispersion of sodium hydride (11 mg, 0.44 mmol). The mixture is stirred at room temperature for 1 h, then poured into saturated $NaHCO_3$ (30 mL) and extracted with CH_2Cl_2 (2 x 30 mL), dried over Na_2SO_4, concentrated, and purified by flash chromatography (25% EtOAc/hexane) to give 98 mg (87%) of 2,6-bis-*O*-(4-methoxybenzyl)-1-deoxy-3,4-*O*-isopropylidene-1-{4-[2-(trimethylsilyl)ethoxymethoxy]phenylthio}-β-D-

galactopyranose **82** as a colorless oil: R_f 0.4 (25% EtOAc/hexane); ^{1}H NMR ($CDCl_3$, 270 MHz,) δ 7.50 (d, J = 8.6 Hz, 2H), 7.36 (d, J = 8.6 Hz, 2H), 7.24 (d, J = 9.6 Hz, 2H), 6.89 (m, 6H), 5.17 (s, 2H, SEM), 4.75 (d, J = 10.9 Hz, 1H, PMB), 4.62 (d, J = 10.9 Hz, 1H, PMB), 4.51 (d, J = 11.2 Hz, 1H, PMB), 4.49 (d, J = 9.5 Hz, 1H, H-1), 4.44 (d, J = 11.2 Hz, 1H, PMB), 4.22 (t, J = 5.6 Hz, 1H, H-3), 4.17 (dd, J = 5.6, 1.7 Hz, 1H, H-4), 3.83 (dt, J = 1.7, 6.0 Hz, 1H, H-5), 3.81 (s, 6H, 2X-OMe), 3.73 (m, 4H), 3.45 (dd, J = 5.6, 9.7 Hz, 1H, H-2), 1.41 (s, 3H, C-CH_3), 1.34 (s, 3H, C-CH_3), 0.94 (t, J = 8.4 Hz, 2H, CH_2-TMS), 0.01 (s, 9H, $SiMe_3$).

To a solution of **82** (53 mg, 0.082 mmol) in 4 mL of MeOH is added *p*-toluenesulfonic acid hydrate (3 mg, 0.016 mmol). The mixture is stirred at room temperature for 9 h, neutralized with pyridine (2 drops), concentrated, and purified by flash chromatography (60% EtOAc/hexane) to give 35 mg (81%) of 2,6-bis-*O*-(4-methoxybenzyl)-1-deoxy-1-(4-hydroxyphenylthio)-β-D-galactopyranose **83** as a colorless oil: R_f 0.4 (60% EtOAc/hexane); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 7.46 (d, J = 8.6 Hz, 2H), 7.34 (d, J = 8.6 Hz, 2H), 7.24 (d, J = 8.6 Hz, 2H), 6.87 (m, 4H), 6.68 (d, J = 8.2 Hz, 2H), 5.65 (br s, 1H), 4.88 (d, J = 10.9 Hz, 1H, PMB), 4.63 (d, J = 10.9 Hz, 1H, PMB), 4.49 (s, 2H, PMB), 4.46 (d, J = 9.9 Hz, 1H, H-1), 3.99 (d, J = 2.3 Hz, 1H, H-4), 3.80 (s, 6H, 2X-OMe), 3.74 (d, J = 5.3 Hz, 2H), 3.56 (m, 2H), 3.53 (t, J =8.9 Hz, 1H), 2.80 (br s, 1H), 2.50 (br s, 1H).

To a solution of **83** (35 mg, 0.066 mmol) in 4 mL of DMF is added K_2CO_3 (15 mg, 0.11 mmol) and 2-(trimethylsilyl)ethyl bromoacetate (25 mg, 0.10 mmol). The reaction is stirred at 45-50 °C for 3 h, concentrated *in vacuo* and purified by flash chromatography (60% EtOAc/hexane) to give 35 mg (89%) of 2-(trimethylsilyl)ethyl 2-{4-[(2,6-bis-*O*-(4-methoxybenzyl)-1-deoxy-β-D-galactopyranosyl)thio]-

phenoxy}acetate **84** as a colorless oil: R_f 0.5 (60% EtOAc/hexane); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 7.53 (d, J = 8.6 Hz, 2H), 7.33 (d, J = 8.6 Hz, 2H), 7.23 (d, J =8.6 Hz, 2H), 6.87 (m, 4H), 6.79 (d, J = 8.6 Hz, 2H), 4.87 (d, J = 10.6 Hz, 1H), 4.63 (d, J = 10.6 Hz, 1H), 4.56 (s, 2H), 4.49 (s, 2H), 4.48 (d, J = 8.9 Hz, 1H, H-1), 4.30 (m, 2H), 3.81 (s, 6H), 3.74 (dd, J = 2.0, 5.0 Hz, 1H), 3.57 (m, 2H), 3.53 (dd, J = 8.9, 8.9 Hz, 1H), 2.69 (br s, 1H), 2.40 (br s, 1H), 1.03 (m, 2H, CH_2-TMS), 0.01 (s, 9H, $SiMe_3$).

To a solution of **84** (141 mg, 0.205 mmol) in 10 mL of CH_2Cl_2 at -78 °C is added N,N-diisopropylethylamine (0.090 mL, 0.51 mmol), acetic anhydride (0.021 mL, 0.23 mmol) and DMAP (4 mg, 0.03 mmol). The solution is stired at -78 °C for 30 min, then diluted with 25 mL of EtOAc and washed with saturated NH_4Cl (25 mL) and saturated $NaHCO_3$ (25 mL). The aqueous layers are back-extracted with EtOAc (25 mL). The organic layers are combined, dried over Na_2SO_4, concentrated and purified by flash chromatography (45% EtOAc/hexane) to give 125 mg (83%) of 2-(trimethylsilyl)ethyl 2-{4-[(3-*O*-acetyl-2,6-bis-*O*-(4-methoxybenzyl)-1-deoxy-β-D-galactopyranosyl)thio]phenoxy}acetate **85** as a colorless oil: R_f 0.5 (50% EtOAc/hexane); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 7.53 (d, J = 8.6 Hz, 2H), 7.24 (m, 4H), 6.87 (m, 4H), 6.79 (d, J = 8.9 Hz, 2H), 4.89 (dd, J = 2.6, 9.5 Hz, 1H, H-3), 4.78 (d, J = 10.5 Hz, 1H), 4.55 (m, 4H), 4.50 (s, 2H), 4.46 (d, J = 11.5 Hz, 1H), 4.30 (m, 2H), 4.18 (dd, J = 2.6, 3.4 Hz, 1H, H-4), 3.81 (s, 3H, OMe), 3.80 (s, 3H, OMe), 3.74 (m, 3H), 3.60 (t, J = 4.6 Hz, 1H), 2.91 (d, J = 3.4 Hz, 1H), 2.06 (s, 3H, OAc), 1.03 (m, 2H, CH_2-TMS), 0.04 (s, 9H, $SiMe_3$).

To a solution of **85** (1.30 g, 1.79 mmol) in 13 mL of CH_2Cl_2 at -35 °C is added pyridine (0.072 mL, 8.93 mmol) followed by triflic anhydride (0.451 mL, 2.68 mmol). The reaction is allowed to warm slowly to 0

°C and is then poured into saturated $NaHCO_3$ (50 mL), extracted with CH_2Cl_2 (3 x 30 mL), dried over Na_2SO_4, concentrated and purified by flash chromatography (25% EtOAc/hexane) to give 1.10 g (71%) of 2-(trimethylsilyl)ethyl 2-{4-[(3-*O*-acetyl-2,6-bis-*O*-(4-methoxybenzyl)-1-deoxy-4-*O*-trifluoromethanesulfonyl-β-D-galactopyranosyl)thio]phenoxy}acetate **86** as a colorless oil: R_f 0.5 (25% EtOAc/hexane); ^1H NMR ($CDCl_3$, 270 MHz) δ 7.48 (d, *J* = 8.9 Hz, 2H), 7.24 (m, 4H), 6.84 (m, 6H), 5.33 (d, *J* = 2.6 Hz, 1H, H-4), 5.00 (dd, *J* = 2.6, 9.6 Hz, 1H, H-3), 4.76 (d, *J* = 10.9 Hz, 1H), 4.40 (m, 5H), 4.31 (m, 3H), 3.81 (m, 1H), 3.81(s, 6H, 2X-OMe), 3.67 (ddd, *J* = 2.3, 9.6, 9.6 Hz, 1H), 3.53 (dd, *J* = 8.7, 8.7 Hz, 1H), 2.03 (s, 3H, OAc), 1.04 (m, 2H, CH_2-TMS), 0.01 (s, 9H, $SiMe_3$).

To a solution of **86** (1.10 g, 1.28 mmol) in 15 mL of DMF is added NaN_3 (831 mg, 12.8 mmol). The reaction is stirred at room temperature for 1.5 h and then poured into water (100 mL), extracted with CH_2Cl_2 (3 x 50 mL), dried over Na_2SO_4, concentrated and purified by flash chromatography (25% EtOAc/hexane) to give 0.96 g (99%) of 2-(trimethylsilyl)ethyl 2-{4-[(3-*O*-acetyl-4-azido-2,6-bis-*O*-(4-methoxybenzyl)-1,4-dideoxy-β-D-glucopyranosyl)thio]phenoxy}acetate **87** as a colorless oil: R_f 0.5 (25% EtOAc/hexane); ^1H NMR ($CDCl_3$, 270 MHz,) δ 7.51 (d, *J* = 8.9 Hz, 2H), 7.23 (m, 4H), 6.91 (d, *J* = 8.9 Hz, 2H), 6.86 (d, *J* = 6.6 Hz, 2H), 6.79 (d, *J* = 8.9 Hz, 2H), 5.13 (dd, *J* = 9.2, 9.9 Hz, 1H, H-3), 4.77 (d, *J* = 10.9 Hz, 1H), 4.51 (m, 6H), 4.30 (m, 2H), 3.82 (s, 3H, OMe), 3.80 (s, 3H, OMe), 3.68 (m, 3H), 3.39 (dd, *J* = 9.4, 9.4 Hz, 1H), 3.31 (m, 1H), 2.00 (s, 3H, OAc), 1.04 (m, 2H, CH_2-TMS), 0.05 (s, 9H, $SiMe_3$).

To a solution of **87** (370 mg, 0.49 mmol) in 10 mL of THF is added tetrabutylammonium fluoride solution (1M in THF, 2.46 mL, 2.46 mmol). The reaction is stirred at room temperature for 30 min and then diluted with EtOAc (25 mL) and washed with 0.5 M HCl

(20 mL) and saturated NaCl (20 mL). The aqueous layer is back-extracted with EtOAc (25 mL) and the organic layers are combined, dried over Na_2SO_4, concentrated, and purified by flash chromatography (0.3% AcOH, 10% MeOH, in EtOAc) to give 250 mg (78%) of 2-{4-[(3-*O*-acetyl-4-azido-2,6-bis-*O*-(4-methoxybenzyl)-1,4-dideoxy-β-D-glucopyranosyl)thio]-phenoxy}acetic acid **88** as a white solid: R_f 0.4 (0.3% AcOH, 10% MeOH/EtOAc); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.51 (d, *J* = 8.8 Hz, 2H), 7.24 (m, 4H), 6.91 (d, *J* = 8.8 Hz, 2H), 6.86 (d, *J* = 8.4 Hz, 2H), 6.76 (d, *J* = 8.8 Hz, 2H), 5.14 (dd, *J* = 9.2, 9.2 Hz, 1H, H-3), 4.76 (d, *J* = 9.6 Hz, 1H), 4.62 (s, 2H), 4.50 (m, 4H), 3.83 (s, 3H, OMe), 3.80 (s, 3H, OMe), 3.73 (m, 2H), 3.64 (dd, *J* = 9.9, 9.9 Hz, 1H), 3.39 (dd, *J* = 9.2, 9.5 Hz, 1H), 3.32 (m, 1H), 2.00 (s, 3H, OAc).

7.30. 2-{4-[(3-*O*-acetyl-4,6-*O*-benzylidene-1-deoxy-2-*O*-(4-methoxybenzyl)-β-D-galactopyranosyl)thio]phenoxy}acetic acid (95)

To a solution of **81**, prepared as above (139 mg, 0.303 mmol) and 2,6-di-*t*-butyl-4-methyl pyridine (187 mg, 0.91 mmol) in 5 mL CH_2Cl_2 are added chlorotriphenylmethane (101 mg, 0.364 mmol) and silver trifluoromethanesulfonate (78 mg, 0.30 mmol). The reaction mixture is stirred at room temperature for 45 min and then is filtered though celite and washed with 10 mL of aqueous $NaHCO_3$. The aqueous solution is extracted with CH_2Cl_2 (2 x 10 mL) and the organic layers are combined and dried over Na_2SO_4, filtered and concentrated. The product is purified by flash chromatography (25% EtOAc/hexane) to give 171 mg (81%) of 1-deoxy-3,4-*O*-isopropylidene-1-{4-[2-(trimethylsilyl)ethoxymethoxy]phenylthio}-6-*O*-triphenylmethyl-β-D-galactopyranose **89** as white solid: R_f 0.2 (25% EtOAc/hexane); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.53-7.40 (m, 8H), 7.31-7.21 (m, 9H), 6.95 (d, *J* = 8.5Hz, 2H), 5.19 (s, 2H), 4.29 (d, *J* = 10.2Hz,

1H, H-1), 4.14 (dd, J = 5.6, 2.0Hz, 1H, H-4), 4.02 (dd, J = 6.9, 5.6Hz, 1H, H-3), 3.73 (m, 3H), 3.56 (dd, J = 9.6, 6.9Hz, 1H, H-6), 3.48 (ddd, J = 8.9, 6.9, 2.0Hz, 1H, H-2), 3.36 (dd, J = 9.6, 5.3Hz, 1H, H-6), 2.37 (d, J = 2.0Hz, 1H, OH), 1.37 (s,3H), 1.31(s, 3H), 0.96 (t, J = 7.2Hz, 2H), 0.00 (s, 9H).

To a solution of **89** (365 mg, 0.521 mmol) and tetrabutylammonium iodide (770 mg, 2.08 mmol) in 5 mL of DMF is added 4-methoxybenzyl chloride (0.422 mL, 4.17 mmol) and sodium hydride (19 mg, 0.782 mmol). The reaction is stirred at room temperature for one hour and then diluted with 50 mL of CH_2Cl_2, washed with brine (3 x 20 mL), dried over Na_2SO_4, filtered, and concentrated to afford a white solid which is purified by flash chromatography (12% EtOAc/hexane) to give 399 mg (93%) of 1-deoxy-3,4-*O*-isopropylidene-2-*O*-(4-methoxybenzyl)-1-{4-[2-(trimethylsilyl)-ethoxymethoxy]phenylthio}-6-*O*-triphenylmethyl-β-D-galactopyranose **90** as a white foam: R_f 0.25 (12% EtOAc/hexane); ^1H NMR ($CDCl_3$, 270 MHz) δ 7.51 (d, J = 8.5Hz, 2H), 7.48-7.45 (m, 6H), 7.34-7.20 (m, 11H), 6.91-6.87 (m, 4H), 5.16 (s, 2H), 4.76 (d, J = 10.9Hz, 1H), 4.62 (d, J = 10.9Hz, 1H), 4.40 (d, J = 9.9Hz, 1H, H-1), 4.19-4.10 (m, 2H), 3.80-3.70 (m, 5H), 3.58-3.53 (m, 2H), 3.45-3.39 (dd, J = 6.3, 9.6Hz, 1H, H-6), 3.36-3.32 (m, 1H), 1.36 (s, 3H), 1.32 (s, 3H), 0.95 (t, J = 8.2Hz, 2H), 0.00 (s, 9H).

To a solution of **90** (399 mg, 0.486 mmol) in 15 mL of methanol is added *p*-toluenesulfonic acid hydrate (69 mg, 0.365 mmol). The reaction mixture is stirred at room temperature for 4.5 h and then quenched by adding solid $NaHCO_3$ and then concentrated and purified by flash chromatography (3% methanol/EtOAc) to give 125 mg (65%) of 1-deoxy-1-(4-hydroxyphenylthio)-2-*O*-(4-methoxybenzyl)-β-D-galactopyranose **91** as a white solid: R_f 0.5 (10% methanol/EtOAc); ^1H NMR (CD_3OD, 270 MHz) δ 7.42-7.38 (m, 4H), 6.88 (d, J = 8.9Hz, 2H), 6.71 (d, J = 8.6Hz,

2H), 4.74 (s, 2H), 4.42 (d, J = 9.5Hz, 1H, H-1), 3.86 (d, J = 2.6Hz, 1H), 3.79 (s, 3H, OCH_3), 3.77-3.70 (m, 2H), 3.61-3.45 (m, 3H).

To a solution of **91** (717 mg, 1.89 mmol) in 100 mL DMF is added benzaldehyde dimethyl acetal (1.4 mL, 9.12 mmol) and *p*-toluenesulfonic acid hydrate (36 mg, 0.189 mmol). The reaction mixture is stirred overnight at room temperature, quenched by adding solid sodium bicarbonate then concentrated *in vacuo*. The residue is purified by flash chromatography (60% EtOAc/hexane) to give 400 mg (43%) of 4,6-*O*-benzylidene-1-deoxy-1-(4-hydroxyphenylthio)-2-*O*-(4-methoxybenzyl)-β-D-galactopyranose **92** as a white solid: R_f 0.2 (60% EtOAc/hexane); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 7.60 (d, J = 8.8Hz, 2H), 7.47-7.33 (m, 7H), 6.88 (d, J = 8.4Hz, 2H), 6.71 (d, J = 8.8Hz, 2H), 5.53 (s, 1H), 5.17 (s, 1H), 4.75 (d, J = 10.3Hz, 1H), 4.64 (d, J = 10.3Hz, 1H), 4.49 (d, J = 9.3Hz, 1H, H-1), 4.37 (dd, J = 12.4, 1.7Hz, 1H, H-6), 4.20 (d, J = 3.6Hz, 1H, H-4), 4.02 (dd, J = 12.4, 1.4Hz, 1H, H-6), 3.80 (s, 3H, OCH_3), 3.76 (td, J = 8.8, 3.7Hz, 1H, H-3), 3.56 (t, J = 9.1Hz, 1H, H-2), 3.49 (m, 1H, H-5), 2.43 (d, J = 8.4Hz, 1H).

To a solution of **92** (400 mg, 0.806 mmol) in 80 mL of dry DMF is added 2-(trimethylsilyl)ethyl bromoacetate (478 mg, 2 mmol) followed by K_2CO_3 (111 mg, 0.806 mmol). The reaction mixture is stirred at 60 °C for 4 hr and then allowed to cool. The solution is diluted with 150 mL of EtOAc and washed with brine (3 x 40 ml), dried over Na_2SO_4, filtered, concentrated and purified by flash chromatography (50% EtOAc/hexane) to give 260 mg (53%) of 2-(trimethylsilyl)ethyl 2-{4-[(4,6-*O*-benzylidene-1-deoxy-2-*O*-(4-methoxybenzyl)-β-D-galactopyranosyl)thio]phenoxy}acetate **93** as a white solid: R_f 0.3 (50% EtOAc/hexane); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 7.6 (d, J = 8.8Hz, 2H), 7.45-7.25 (m, 7H), 6.82 (d, J = 8.5Hz, 2H), 6.71 (d, J = 8.8Hz, 2H),

5.49 (s, 1H), 4.68 (d, J = 10.3Hz, 1H), 4.57 (d, J = 9.9Hz, 1H), 4.49 (s, 2H), 4.46 (d, J = 9.5Hz, 1H, H-1), 4.32 (dd, J = 12.5, 1.5Hz, 1H, H-6), 4.28-4.22 (m, 2H), 4.16 (d, J = 3.3Hz, 1H, H-4), 3.97 (dd, J = 12.5, 1.5Hz, 1H, H-6), 3.75 (s, 3H, OCH_3), 3.71 (td, J = 8.4, 3.3Hz, 1H, H-3), 3.52 (t, J = 9.1Hz, 1H, H-2), 3.44 (m, 1H, H-5), 2.35 (d, J = 8.4Hz, 1H, OH), 0.989-0.917 (m, 2H), 0.0 (s, 9H).

To a solution of **93** (260 mg, 0.41 mmol) in 50 mL of CH_2Cl_2 is added pyridine (0.140 mL, 1.64 mmol) and acetic anhydride (0.078 mL, 0.82 mmol). The reaction mixture is stirred at room temperature for 2 hr and quenched by adding 1 mL of methanol. The reaction mixture is washed with 20 mL of $NaHCO_3$ solution and the aqueous layer is extracted with CH_2Cl_2 (2 x 40 mL). The organic layers are combined, dried over Na_2SO_4, filtered, concerntrated and purified by by flash chromatography (50% EtOAc/hexane) to give 252 mg (91%) of 2-(trimethylsilyl)ethyl 2-{4-[(3-*O*-acetyl-4,6-*O*-benzylidene-1-deoxy-2-*O*-(4-methoxybenzyl)-β-D-galactopyranosyl)thio]-phenoxy}acetate **94** as a white solid: R_f 0.4 (50% EtOAc/hexane); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 7.63 (d, J = 8.9Hz, 2H), 7.48-7.24 (m, 7H), 6.86 (d, J = 8.6Hz, 2H), 6.72 (d, J = 8.9Hz, 2H), 5.49 (s, 1H), 4.92 (dd, J = 9.9, 3.3Hz, 1H, H-3), 4.70 (d, J = 10.2Hz, 1H), 4.58 (d, J = 9.6Hz, 1H, H-1), 4.51 (s, 2H), 4.46 (d, J = 10.2Hz, 1H), 4.38-4.27 (m, 4H), 4.00 (bd, J = 12.2Hz, 1H, H-6), 3.84 (t, J = 9.5Hz, 1H, H-2), 3.79 (s, 3H, OCH_3), 3.55 (m, 1H, H-5), 2.03 (s, 3H), 1.07-1.00 (m, 2H), 0.05 (s, 9H).

To a solution of **94** (252 mg, 0.365 mmol) in 2 mL of THF is added tetrabutylammonium fluoride solution (1.0M in THF, 0.380 ml, 0.380 mmol) at room temperature. The reaction mixture is stirred for 5 mins and then directly loaded to a silica gel column and purified by by flash chromatography (0.1% AcOH,10% methanol, in EtOAc) to give 196 mg (91%) of

the title compound **95** as a white solid: R_f 0.1 (0.1% AcOH,10% methnol/EtOAc); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 9.72 (bs, 1H), 7.57 (d, *J* = 8.5Hz, 2H), 7.47-7.29 (m, 5H), 7.26 (d, *J* = 8.5Hz, 2H), 6.86 (d, *J* = 8.4Hz, 2H), 6.83 (d, *J* = 8.4Hz, 2H), 5.48 (s, 1H), 4.92 (dd, *J* = 9.5, 3.3Hz, 1H, H-3), 4.73 (d, *J* = 11.2Hz, 1H), 4.57 (d, *J* = 9.5Hz, 1H, H-1), 4.50-4.38 (m, 3H), 4.37-4.31 (m, 2H), 4.00 (bd, *J* = 12Hz, 1H, H-6), 3.85 (t, *J* = 9.5Hz, 1H, H-2), 3.80 (s, 3H, OCH_3), 3.52 (m, 1H, H-5), 2.03 (s, 3H).

7.31 Attachment Of A Glycosyl Acceptor To A Resin Support

TentaGel S NH_2 resin (0.500 g) is suspended in N-methylpyrrolidinone (NMP, 15 mL), and to this mixture is added acid **64** (0.115 g, 0.230 mmol), diisopropylethylamine (0.22 g, 1.3 mmol), and HOBT/HBTU solution (0.45 M in DMF, 2.2 g, 0.93 mmol). The reaction mixture is then shaken for 2-5 h until the resin gives a negative Kaiser test. The resin is washed with CH_2Cl_2 (3 x 15 mL, 5 min), NMP (3 x 15 mL, 5 min), and DMF (3 x 15 mL, 5 min).

A solution of hydrazine in DMF (1:7 v/v, 24 mL) is added to the resin. The mixture is shaken for 9 h or until acetate hydrolysis is shown to be complete by IR analysis (KBr pellet). The resin is then washed with DMF (3 x 15 mL, 5 min), H_2O (3 x 15 mL, 5 min), methanol (3 x 15 mL, 5 min) and CH_2Cl_2 (3 x 15 mL, 5 min).

In the present embodiment, six glycosyl acceptors (glycosylated acids **64**, **66**, **76**, **77**, **88** and **95**) are attached to six separate batches of resin (e.g., TentaGel) and deprotected using the procedure described above. Afterwards, 0.450-gram portions of each resin are combined, suspended in 15 mL of CH_2Cl_2, shaken for 15 min and dried on the lyophilizer for 12 h.

7.32. Procedure For Solid Phase Glycosylation

Predetermined portions (0.225 g) of the mixed resin are placed in twelve glycosylation reaction vessels equipped with glass-fritted bottoms, suspended in 5 mL of CH_2Cl_2 and agitated by slowly bubbling argon through the glass frit for 10 min. Each of the twelve resin samples is then glycosylated as follows: The 1-sulfinyl hexose derivative **5** (0.137 g, 0.270 mmol) and 2,6-di-*tert*-butyl-4-methylpyridine (0.111 g, 0.540 mmol) are dissolved in toluene (10 mL). The toluene is subsequently removed *in vacuo* to remove any water that may be present in solution. The dissolution and drying step is repeated at least once more. The residue is then dried under vacuum for 1 h. Afterwards, the sulfoxide and base are dissolved in CH_2Cl_2 (10 mL) and added to the first resin sample. The suspension is then cooled to -65 °C, and a solution of trifluoromethanesulfonic anhydride (23 µL, 0.13 mmol) in 1 mL of CH_2Cl_2 is added dropwise over 10 min. The reaction mixture is allowed to warm to 0 °C over 1-2 h, quenched using saturated aqueous $NaHCO_3$ (10 mL) and agitated for 10 min. The resin is then washed sequentially with 10 mL portions (3 x 10 mL, 5 min for each portion) of the following solvents: $NaHCO_3$, H_2O, methanol, diethyl ether, CH_2Cl_2 and toluene. The resin is dried on the lyophilizer (in vacuo) for 12 h and resubjected to the glycosylation reaction conditions for a second time to ensure complete reaction.

Thus, using the above-described procedure, the twelve resin samples are glycosylated with the 1-sulfinyl hexose derivatives **5**, **11**, **13**, D-**25**, L-**25**, **33**, **37**, **41**, D-**45**, L-**45**, **51** and **53**. All 12 portions of the resin are then combined, suspended in 15 mL of CH_2Cl_2, shaken for 15 min and dried on the lyophilizer for 12 h.

7.33. Azide Reduction And Amine Acylation

Predetermined portions (0.139 g) of the resin are placed in 19 reaction vessels. Eighteen of the resin portions are subjected to the following reduction conditions to convert the azide groups to amino groups: The resin is suspended in anhydrous THF (8 mL), treated with trimethylphosphine (1.0 M solution in THF, 0.5 mL, 0.5 mmol) and shaken at room temperature for 4 h. To the suspension is added 1 mL of H_2O, and the reaction vessel is shaken for an additional 37 h at 48 °C. The resin is washed with THF (3 x 15 mL, 5 min) to remove the trimethylphosphine. The resin is then resuspended in THF (8 mL) and water (0.5 mL) and heated at 70-75 °C for 24 h to hydrolyze the ylide completely. Afterwards, the resin is washed sequentially with THF (3 x 15 mL, 5 min) CH_2Cl_2 (3 x 15 mL, 5 min) and then dried on the lyophilizer for 12 h.

Seventeen of the resulting resin samples are then derivatized at the pendant amino group according to one of two different procedures. Typical conditions for the coupling of pyridine-4-carboxylic acid, pyridine-4-carboxylic acid N-oxide, N-acetyl-D-alanine and N-acetyl-L-alanine (the acids **14**, **15**, **17** and **18**, respectively) are as follows: The resin is suspended in a solution of 1:1 DMF/CH_2Cl_2 (6 mL). Then one of the above-mentioned acids (0.42 mmol) and diisopropyl carbodiimide (66 µL, 0.052 g, 0.42 mmol) are added. The reaction mixture is shaken at room temperature for 12-24 h or until the resin gives a negative Kaiser test. The resin is washed sequentially (3 x 8 mL portions, 5 min for each portion) with the following solvents: DMF, isopropanol and CH_2Cl_2.

Typical conditions for the coupling of methanesulfonyl chloride, methyl isocyanate, methyl

isothiocyanate, 3-methylbutyryl chloride, pentanoyl chloride, methyl chloroformate, benzoyl chloride, 4-nitrobenzoyl chloride, 2-thiophenecarbonyl chloride, 2-iodobenzoyl chloride and glutaric anhydride are as follows: The resin is suspended in CH_2Cl_2 (6 mL), and to the suspension is added triethylamine (70 µL, 0.50 mmol) or diisopropylethylamine (88 µL, 0.50 mmol). In addition, DMAP (0.010 g, 0.080 mmol) is added in the cases of acetic anhydride and diketene. One of the acylating reagent listed above (0.42 mmol) is then added, and the reaction mixture is shaken at 4 °C for 12 h. The acylation reactions for reagents methyl isocyanate and methyl isothiocyanate are conducted at 50 °C for 12 h. All resin portions give a negative Kaiser test, thus indicating a complete reaction. The resin is next washed with CH_2Cl_2 (3 x 8 mL, 5 min for each portion).

7.34. Deprotection Of Resin-bound Disaccharide

Predetermined portions (0.050 g) of each of the twenty resin samples prepared above are combined, suspended in 15 mL of CH_2Cl_2, shaken for 15 min, and drained. A solution of 20% trifluoroacetic acid (TFA) in CH_2Cl_2 (18 mL) is added to the resin and shaken at room temperature for 30 min. The resin is washed with CH_2Cl_2 (3 x 18 mL, 5 min for each portion) and dried in vacuo for 12 h.

The resin is suspended in a solvent mixture of THF:MeOH (1:4 v/v, 20 mL) for 10 min, and ground $LiOH \cdot H_2O$ (0.20 g, 4.8 mmol) is added. The reaction mixture is shaken at room temperature for 12 h. The resin is then washed with H_2O until the pH of the filtrate is determined to be neutral. The neutral resin is then dried in vacuo for 12 h.

7.35. Test For L ctin-Affinity

The biotin labeled lectin from *Bauhinia purpurea* (Camels foot tree) has been described

(Makela, O. and Makela, P. *Ann. Med. Exp. Fenn.* (1956) 84:402 and Osawa, T. et al. *Methods in Enzymology* (1978) 50:367. To test or probe the disaccharide library for lectin binders, a sample of the resin beads (10 mg) is washed three times for 10 min each with 1 mL of PBST buffer (10 mM sodium phosphate, pH 7.2/150 mM NaCl/0.05% Tween-20). The resin sample is then incubated for 30 min at room temperature in 1 mL of PBST containing 3% bovine serum albumin (BSA) to block any nonspecific protein binding sites and then washed three times for 5 min each with 1 mL of PBST containing 1% BSA. The resin sample is incubated in 1 mL of a lectin solution (10 μg/mL in PBST containing 1% BSA) at room temperature for 3 h. The resulting resin sample is then washed three times for 5 min each with 1 mL of TBST buffer (20 mM Tris·HCl, pH 7.5/500 mM NaCl/0.05% Tween-20) containing 1% BSA.

The resin sample is incubated for 20 min at room temperature in 1 mL of alkaline phosphatase-coupled streptavidin (10 μg/mL in TBST containing 1% BSA). After the resin sample is washed three times for 5 min each with 1 mL of alkaline phosphatase buffer (100 mM Tris·HCl, pH 9.2/100 mM NaCl/5 mM $MgCl_2$), it is then divided into three equal portions (~3 mg each). Each portion is incubated for 20 min in 200 μL of a commercial BCIP/NBT solution (5-bromo-4-chloro-3-indoyl phosphate and nitro blue tetrazolium; See, e.g., Blake, M. *Anal. Biochem.* (1984) 136:175). To stop the reaction, the resin is washed twice with 200 μL of 20 mM NaEDTA, pH 7.4. When viewed under a low-power microscope during the staining period, dark purple beads are easily distinguished from the vast majority of nearly colorless beads. It is also noted that the purple color on the beads develops at different rates.

Out of the total sample (approximately 8,000 beads), eight beads are observed to stain dark purple

within five minutes, nine beads stain purple after ten minutes and another twenty-one beads are selected that stain a pale purple after about twenty minutes. (See, Fig. 15.)

7.36. Structural Determination Of Binders Detected

Each of the thirty-eight purple beads, identified above by their color as having bound to the lectin probe, is treated to release the saccharide-containing moiety from the solid support. The released moiety is subsequently analyzed by a variety of methods, including but not limited to, GC-mass spectroscopy, GC-Fourier Transform Spectrophotometry, nuclear magnetic resonance spectroscopy and the like. Knowing the starting reagents used in building the combinatorial library and the sequence in which each is used, the identity of each binding moiety is determined from the additional analytical data (such as mass spectral data) obtained above.

Other embodiments should be apparent to those of ordinary skill in view of the detailed disclosure provided herein, which embodiments would nonetheless fall within the scope and spirit of the present invention. For example, any compound or substance, including but not limited to small molecules, purines, pyrimidines, nucleosides, nucleic acids, other sugars, amino acids, peptides, proteins, other natural polymers, unnatural polymers, synthetic polymers and the like, having a nucleophilic group capable of forming a covalent bond with a glycosyl donor can be utilized in the present invention. Hence, the preceding preferred embodiments should not be construed as limiting the invention in any way.

WHAT IS CLAIMED IS:

1. A library comprising a collection of distinct carbohydrate-based ligands, a plurality of each ligand being bound to and presented on the surface of a resolvable portion of a solid support to permit: (i) multivalent interactions of said plurality of ligands with one or more probes bearing a plurality of carbohydrate binding sites, and (ii) selection of at least one particular ligand-probe interaction,

which library is prepared by a method comprising a glycosyl bond-forming step.

2. The library of claim 1 in which said solid support comprises a planar support, separate wells, a multi-well microtiter plate, a spherically shaped bead.

3. The library of claim 1 in which said solid support comprises a plurality of solid or porous beads.

4. The library of claim 1 in which said solid support comprises a polyether chain-modified polystyrene.

5. The library of claim 1 in which said probe comprises one or more receptors comprising a peptide or a protein.

6. The library of claim 1 in which said probe comprises an intact cell or a portion thereof.

7. The library of claim 6 in which said cell is selected from among those involved in a cell-mediated immune response.

8. The library of claim 6 in which said cell is selected from among those involved in the production of antibodies.

9. The library of claim 1 in which the glycosyl bond-forming step includes a condensation reaction between a glycosyl donor (GD) and a solid support-bound glycosyl acceptor (GA-SS) to provide a structural unit (GD-GA-SS) with a newly formed glycosyl bond.

10. The library of claim 9 in which the glycosyl bond-forming step includes a plurality of condensation reactions taking place substantially concurrently between a glycosyl donor and a plurality of distinct solid support-bound glycosyl acceptors to provide a plurality of distinct structural units with newly formed glycosyl bonds.

11. The library of claim 9 in which the glycosyl bond-forming step includes a plurality of condensation reactions taking place substantially concurrently between a plurality of distinct glycosyl donors and a solid support-bound glycosyl acceptor to provide a plurality of distinct structural units with newly formed glycosyl bonds.

12. The library of claim 9 in which the glycosyl bond-forming step includes a plurality of condensation reactions taking place substantially concurrently between a plurality of distinct glycosyl donors and a plurality of distinct solid support-bound glycosyl acceptors to provide a plurality of structural units with newly formed glycosyl bonds.

13. The library of claim 1 in which the glycosyl bond-forming step includes a sulfoxide-mediated glycosylation reaction.

14. An assay for a carbohydrate-based ligand-receptor interaction comprising:

(a) providing a library comprising a collection of distinct carbohydrate-based ligands, a plurality of each ligand being bound to and presented on the surface of a resolvable portion of a solid support;

(b) contacting said library with one or more probes bearing a plurality of carbohydrate binding sites; and

(c) selecting at least one particular ligand-probe interaction.

15. The assay of claim 14 in which the selection step includes selecting those resolvable portions of the solid support to which a probe has bound.

16. The assay of claim 14 in which said library permits multivalent interactions of said plurality of ligands with said one or more probes.

17. The assay of claim 14 in which said solid support comprises a polyethylene resin, a poly(ethylene glycol) resin, or a dendrimer polymer.

18. A carbohydrate-based ligand selected by the method of claim 14.

19. The ligand of claim 18 which is an enzyme inhibitor, a receptor agonist, a receptor antagonist, an antigen, an immunogen, an anti-tumor agent, an anticancer agent, an anti-emetic agent, an anti-inflammatory agent, a neurotransmitter, or a substance that exhibits endocrine-like properties.

20. A method of preparing a library comprising

a collection of distinct carbohydrate-based ligands each bound to a resolvable portion of a solid support (SS) comprising (a) providing a plurality of distinct solid support-bound glycosyl acceptors (GA_1-SS, GA_2-SS, etc.), each distinct solid support-bound glycosyl acceptor being bound to a resolvable portion of a solid support, (b) contacting said plurality of distinct solid support-bound glycosyl acceptors with at least one distinct glycosyl donor (GD) such that condensation reactions take place, including glycosyl bond-forming steps, between said at least one distinct glycosyl donor and each of said distinct solid support-bound glycosyl acceptors to provide at least the distinct structural units (GD-GA_1-SS, GD-GA_2-SS, etc.).

21. The method of claim 20 in which said plurality of distinct solid support-bound glycosyl acceptors is provided in separate reaction vessels each holding a distinct solid support-bound glycosyl acceptor.

22. The method of claim 21 in which at least one distinct glycosyl donor is contacted with each of said distinct solid support-bound glycosyl acceptors.

23. The method of claim 20 in which said plurality of distinct solid support-bound glycosyl acceptors is not provided in separate reaction vessels each holding a distinct solid support-bound glycosyl acceptor.

24. The method of claim 23 in which at least one distinct glycosyl donor is contacted with said plurality of distinct solid support-bound glycosyl acceptors substantially concurrently in the same reaction vessel.

25. The method of claim 20 which further comprises contacting at least the distinct structural units (GD-GA_1-SS, GD-GA_2-SS, etc.) with one or more additional reagents, including one or more additional glycosyl donors.

26. A composition for use as a vaccine comprising a plurality of one or more distinct carbohydrate-based ligands and, optionally, one or more distinct non-carbohydrate-based ligands, which carbohydrate-based ligands at least are bound to and presented on the surface of a solid support to permit the multivalent interaction of said plurality of one or more distinct carbohydrate-based ligands with one or more receptors associated with an immune system response,

such that an individual, to whom an effective amount of said composition has been administered, is able to mount an appropriate immune response against a given disease that is caused by a given pathogen or which is characterized by the expression of a given marker on the surface of a cell affected by said disease.

27. The composition of claim 26 which further comprises a pharmaceutically acceptable carrier.

28. The composition of claim 26 in which said carrier enhances the immunogenic response to the composition.

29. The composition of claim 26 in which said optional non-carbohydrate-based ligands are selected from small molecules, drugs, peptides, glycopeptides, deoxyribonucleic acids, ribonucleic acids, lipids, or combinations or complexes thereof.

30. A method of immunizing an individual

comprising administering to an individual in need of immunization an effective amount of vaccine comprising a plurality of one or more distinct carbohydrate-based ligands and, optionally, one or more distinct non-carbohydrate-based ligands, which carbohydrate-based ligands at least are bound to and presented for multivalent interaction on a scaffold or on the surface of a solid support.

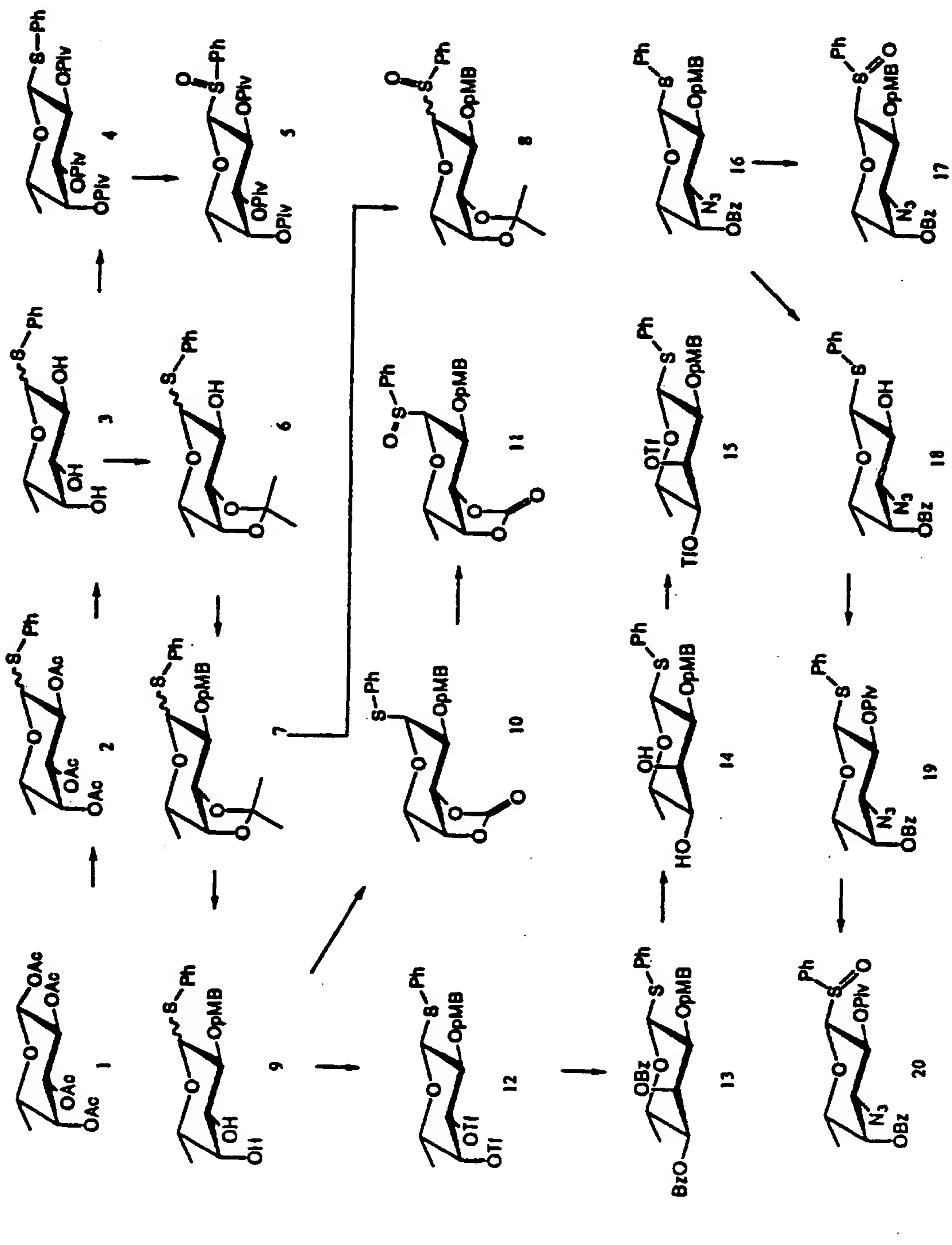


Figure 1

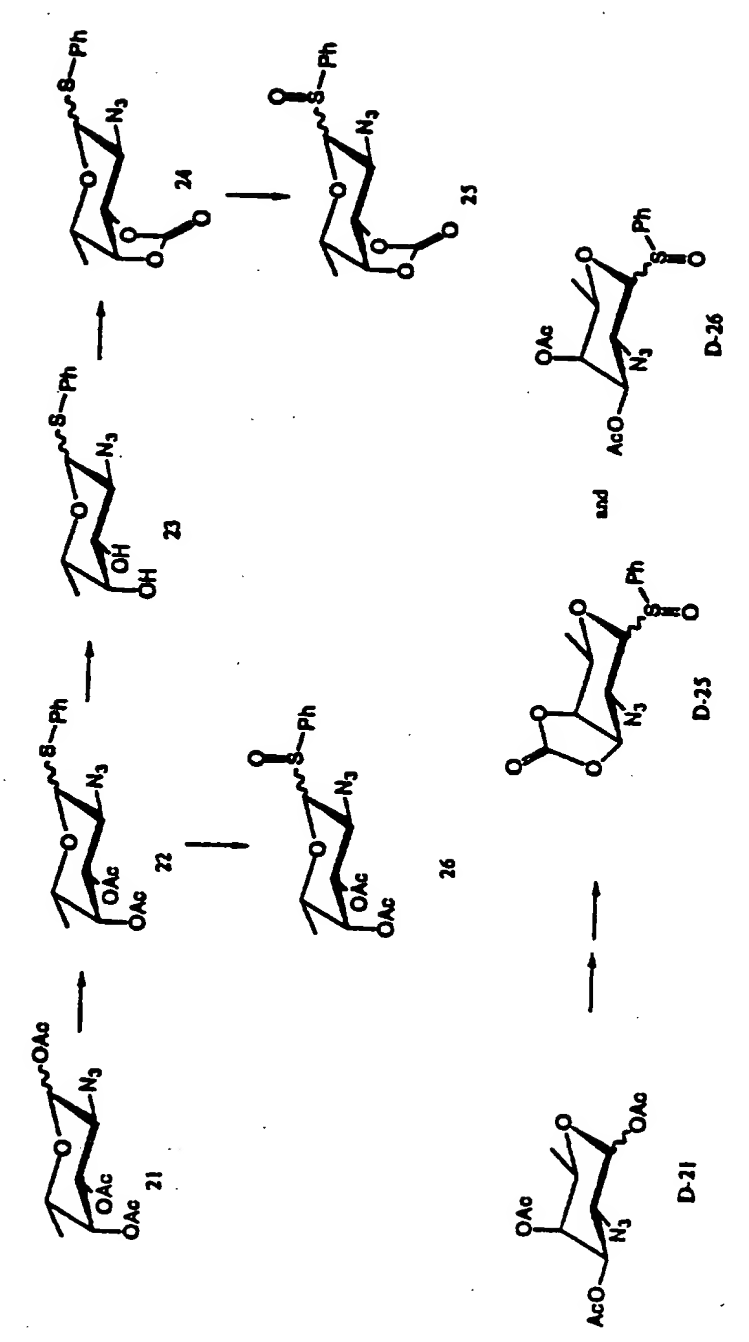


Figure 2

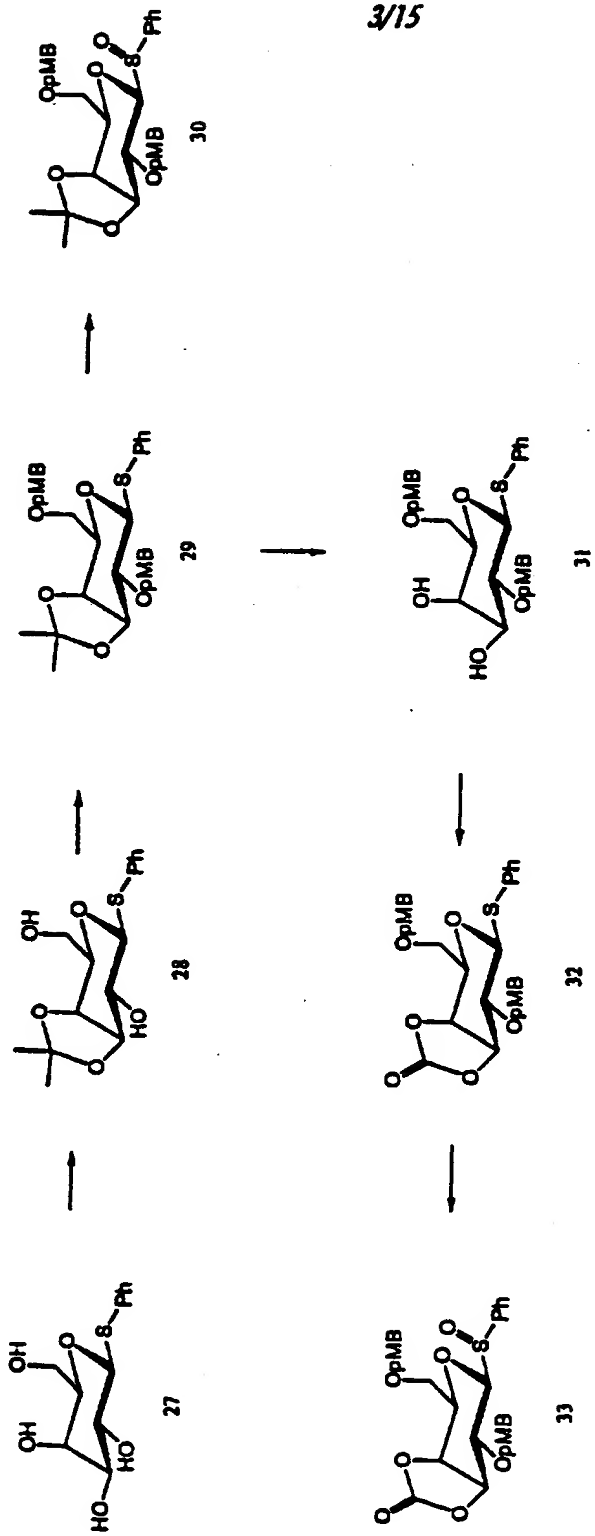


Figure 3

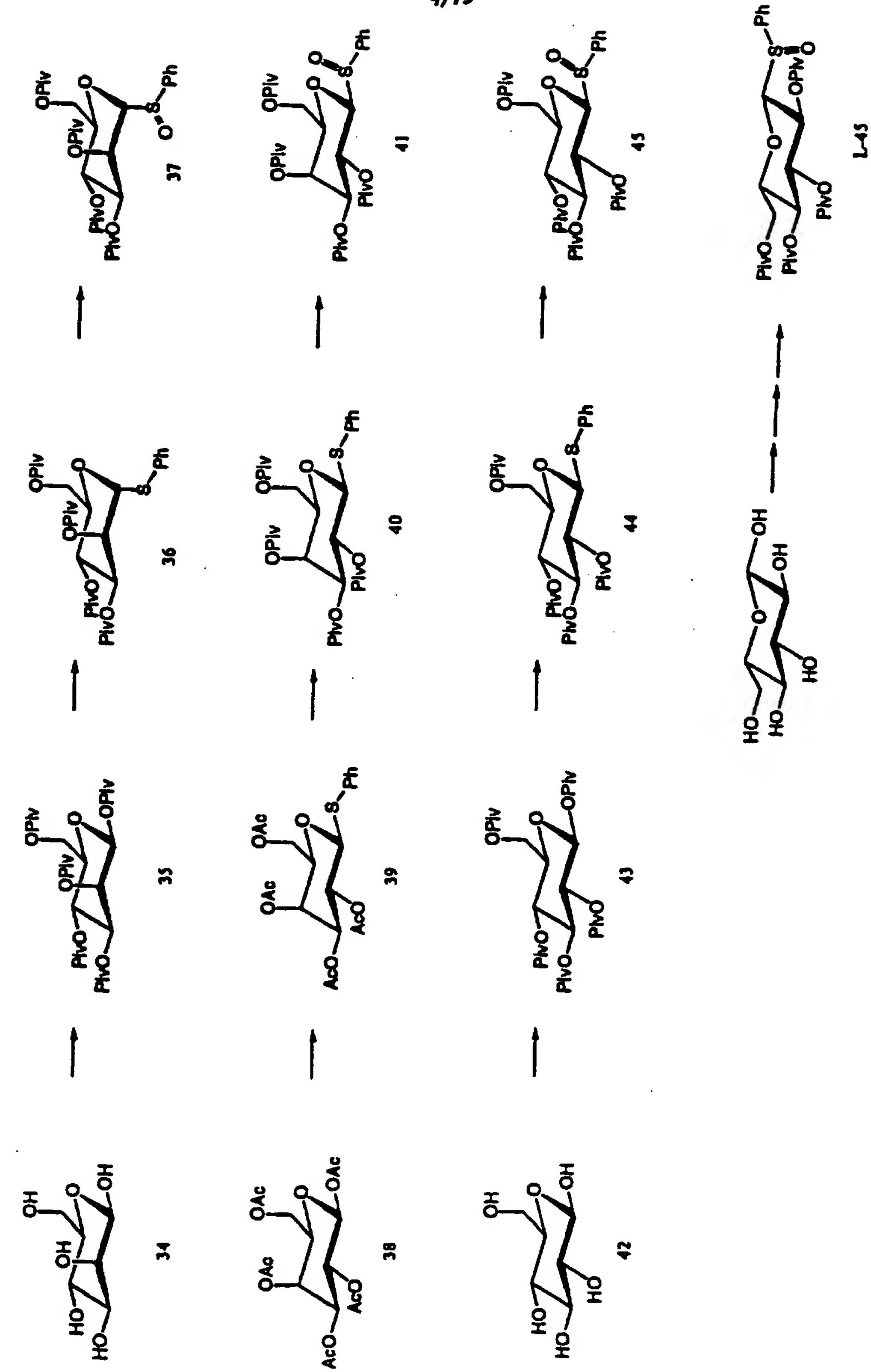


Figure 4

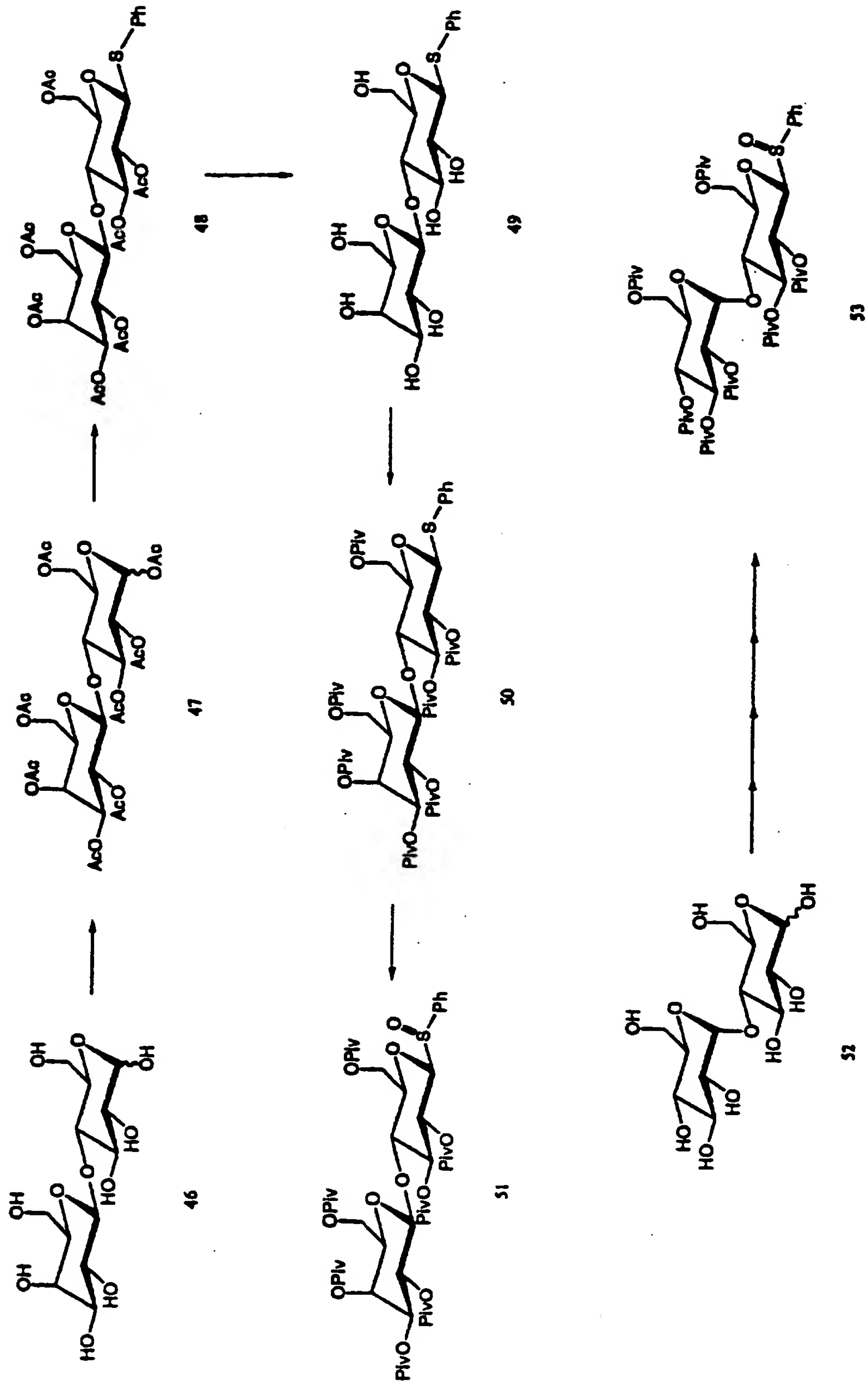


Figure 5

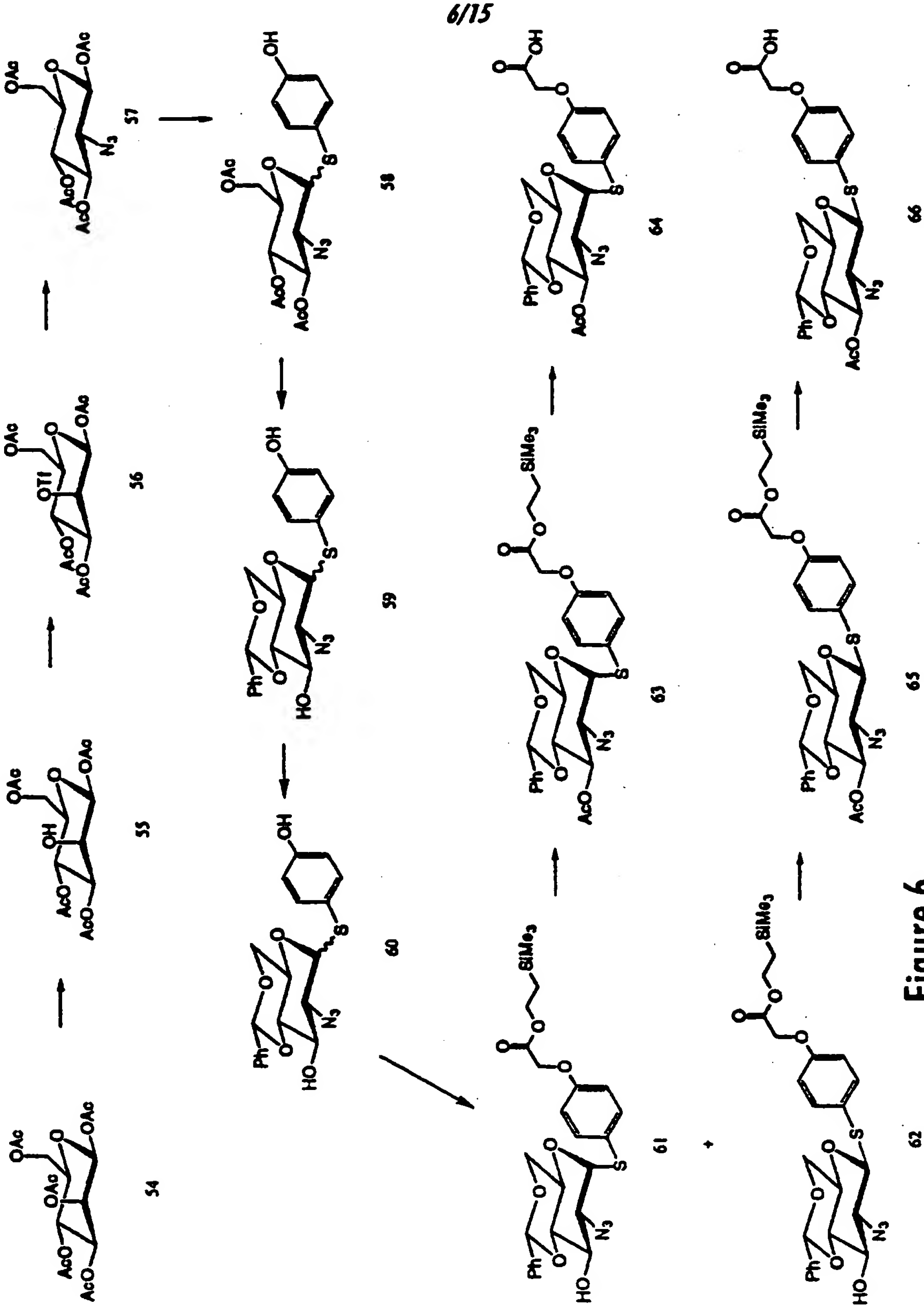


Figure 6

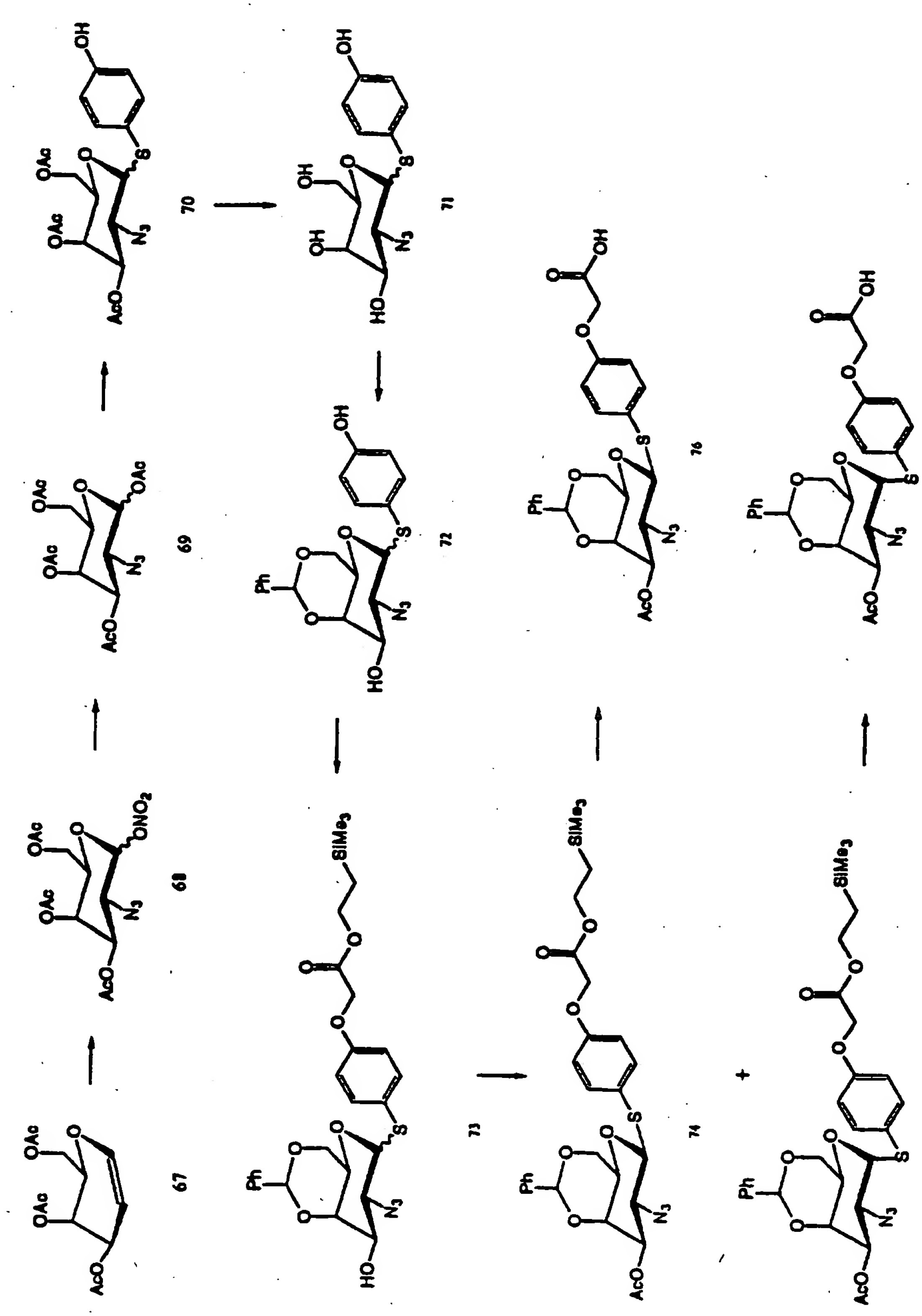


Figure 7

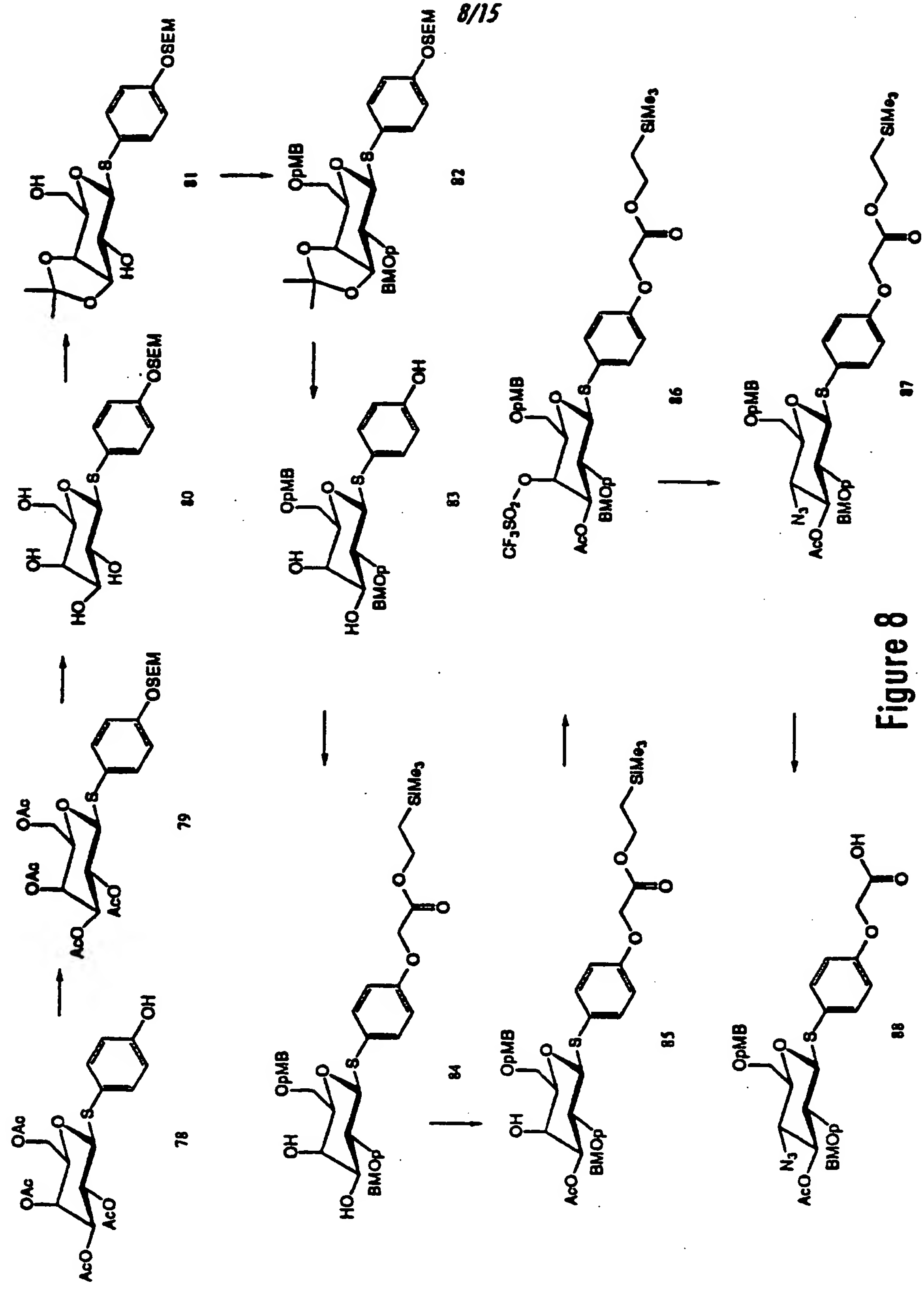


Figure 8

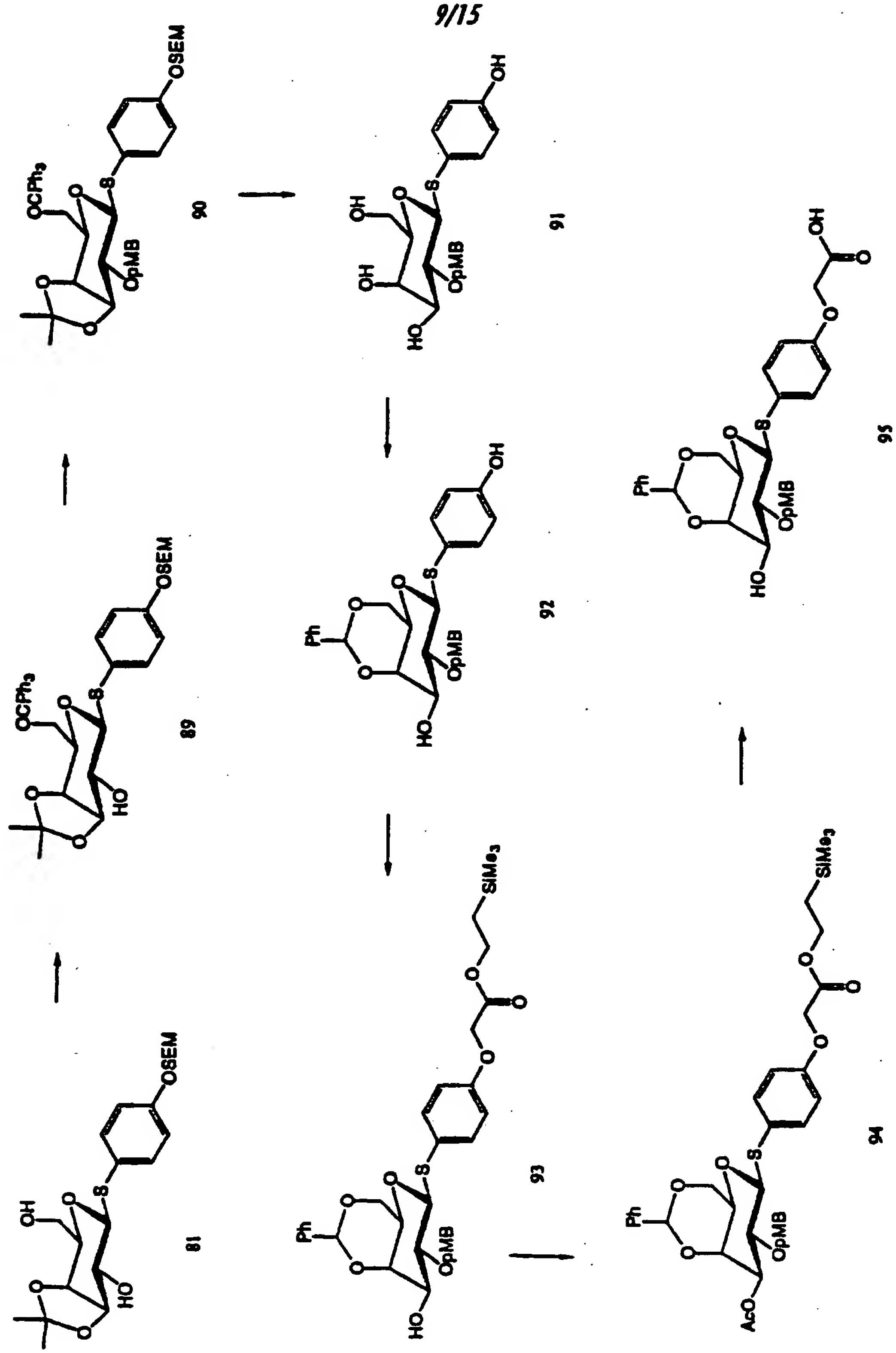
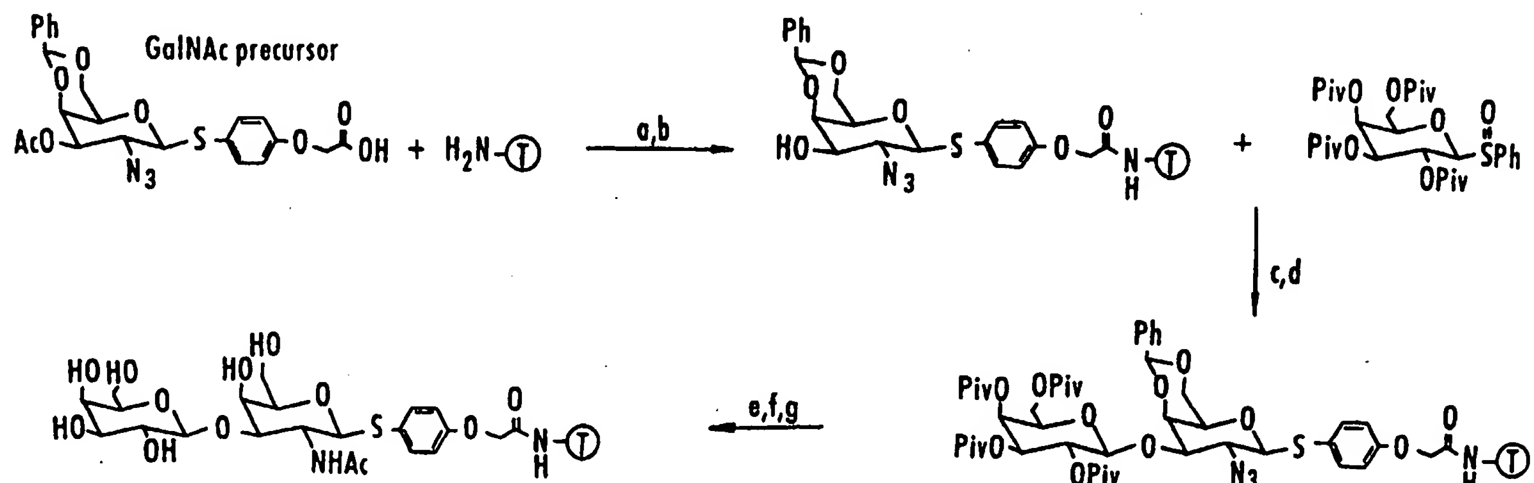


Figure 9



Conditions: (a) HOBT/HBTU,DIEA, NMP, rt, 2h; (b) NH_2NH_2, DMF (1:7), rt, 6h; (c) Tl_2O, DTBMP, CH_2Cl_2, -60 to 0 °C, 1.5h; (d) repeat; (e) thioacetic acid, rt, 2h; (f) 20% TFA, CH_2Cl_2, rt, 30 min; (g) LIOH, MeOH/THF, rt, 10h

GlcNAc precursor

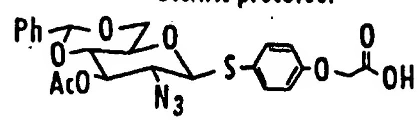


Figure 10

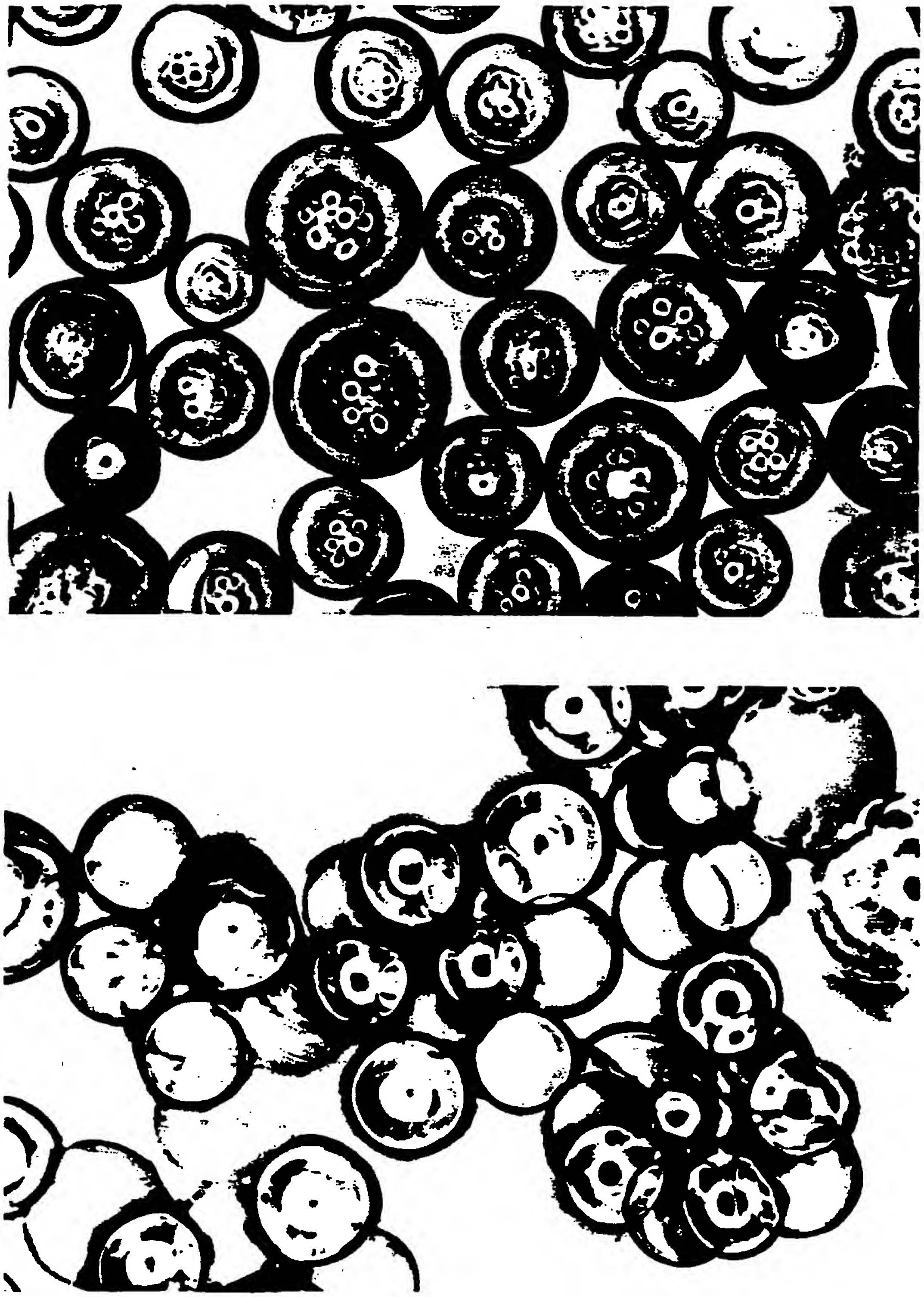


Figure 11

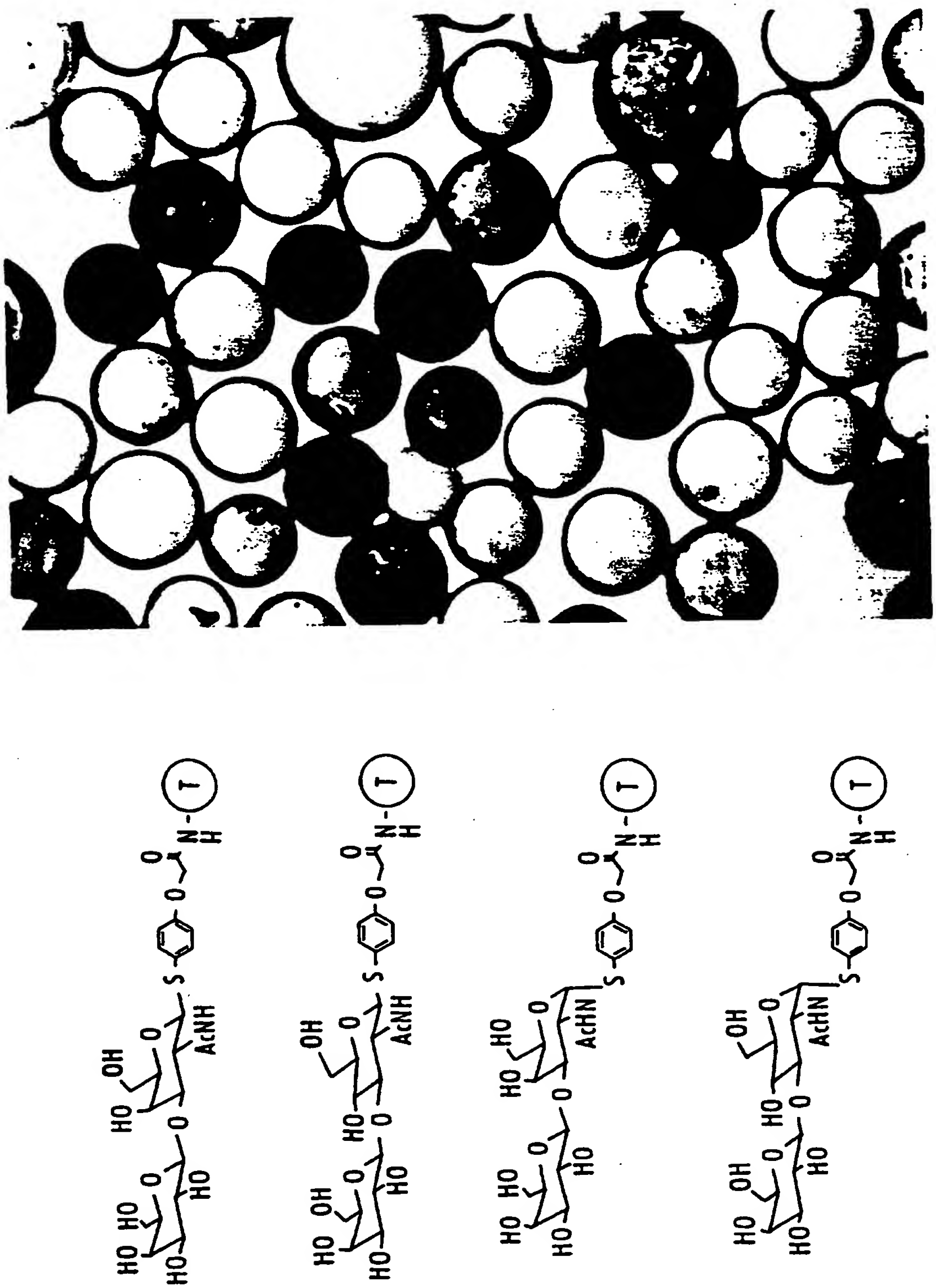
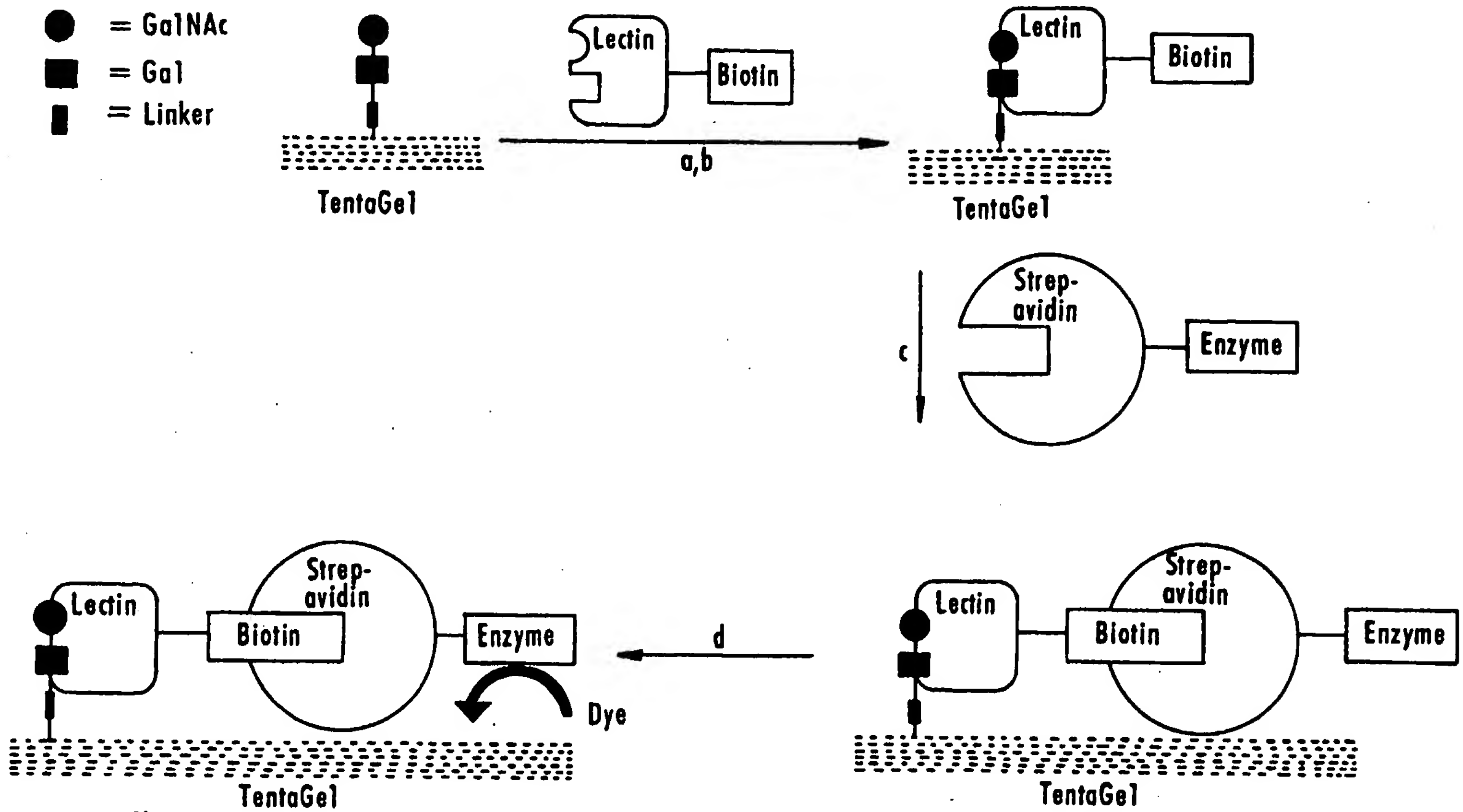


Figure 12



Clorimetric assay of carbohydrate binding: (a) 3% BSA (bovine serum albumin)/PBST (10mM sodium phosphate, pH 7.2; 150 mM NaCl; 0.05% Tween-20), 30 min; (b) 10ug/ml of biotin-labelled Bauhinia Purpurea in 1 % BSA/PBST, 3 h; (c) 10 ug/ml alkaline phosphatase-coupled strepavidin in 1 % BSA/TBST (20 mM Tris HCl, pH 7.5; 500 mM NaCl; 0.05% Tween-20); (d) BCIP (5-bromo-4-chloro-3-indolyl phosphate/NBT (nitroblue tetrazolium)

Figure 13

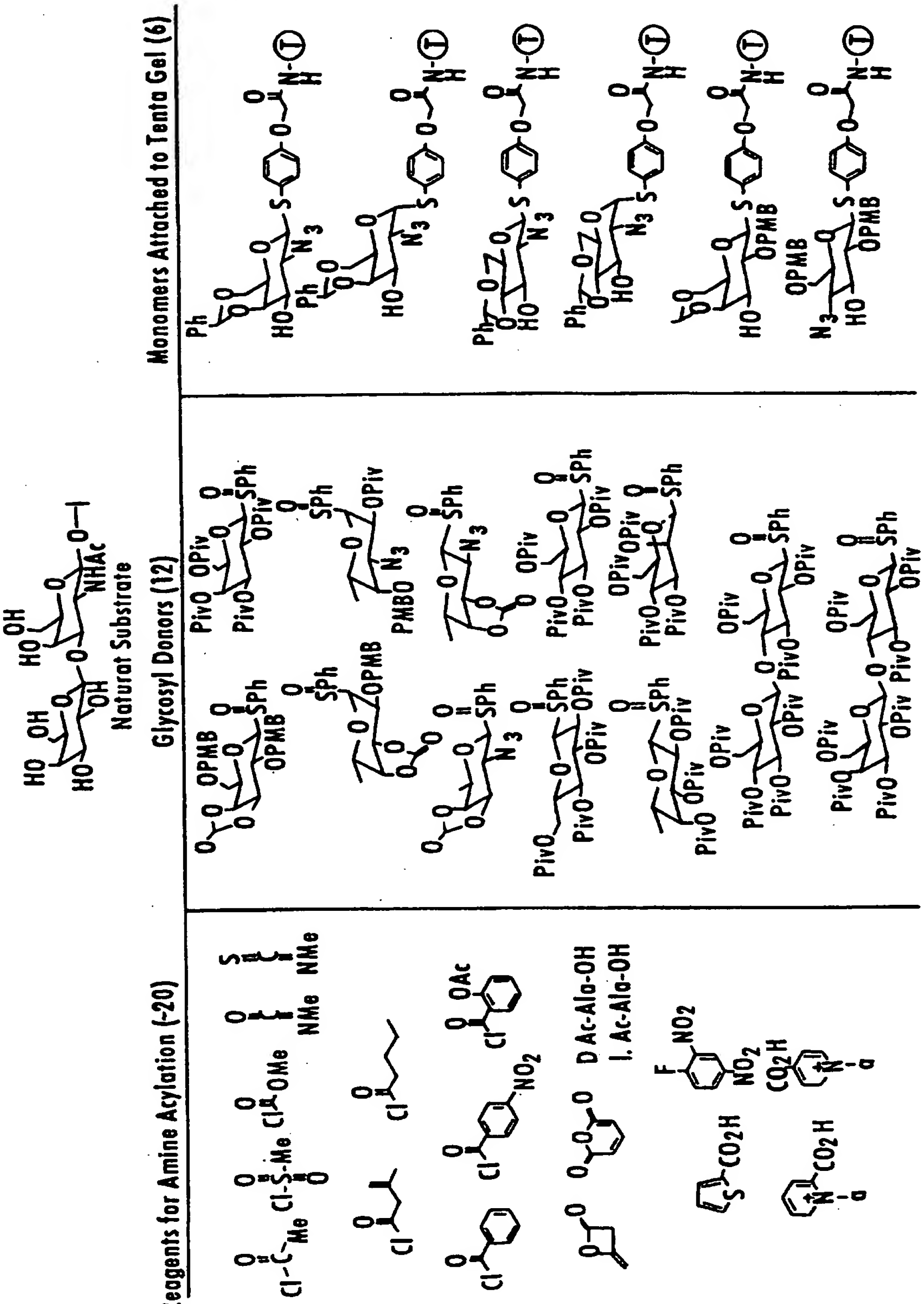

Natural Substrate
Reagents for Amine Acylation (~20)
Glycosyl Donors (12)
Monomers Attached to Tenta Gel (6)
D Ac-Ala-OH
L Ac-Ala-OH

Figure 14

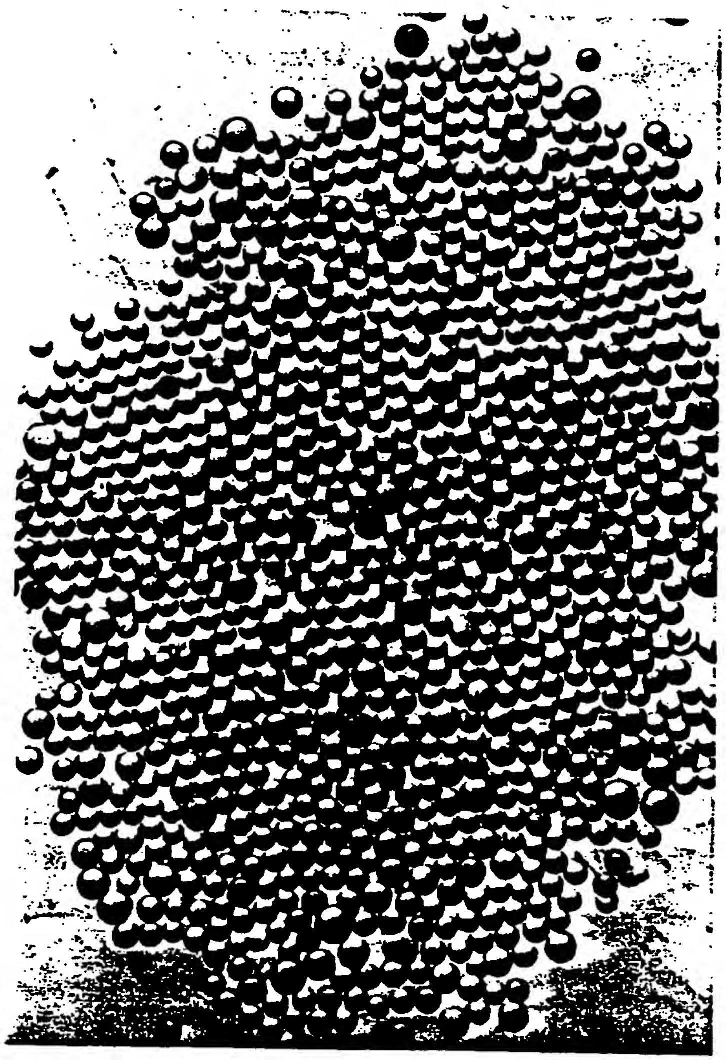


Figure 15

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/04639

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :G01N 33/543; C12N 9/00; C07H 5/08, 15/12, 15/14; A61K 39/385
US CL :436/518; 435/183; 536/4.1, 18.5, 18.6; 424/184.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 436/518; 435/183; 536/4.1, 18.5, 18.6; 424/184.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, Dialog

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y, P	US 5,575,324 A (STILL et al.) 15 October 1996, see entire document.	1-30
Y,P	US 5,510,240 A (LAM et al.) 23 April 1996, see entire document.	1-30
Y	WO 95/03315 A2(OXFORD GLYCOSYSTEMS LTD) 02 February 1995, see entire document.	1-30
Y	WO 95/18971 A1 (AFFYMAX TECHNOLOGIES N.V.) 13 July 1995, see entire document.	1-30
X,P	LIANG, R. et al. Parallel Synthesis and Screening of a Solid Phase Carbohydrate Library. Science. November 1996. Vol. 274. pages 1520-1522, see entire document.	1-30

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 JUNE 1997	10.07.1997

Name and mailing address of the ISA/US	Authorized officer
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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	OHLMEYER, M. et al. Complex synthetic chemical libraries indexed with molecular tags. Proc. Natl. Acad. Sci. USA. December 1993. Vol. 90. pages 10922-10926. see entire document.	1-30

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/04639

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
Claims 1-18, 19 (in part), 20-30

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.